

Standardization of a Crude Drug *Moringa oleifera* Leaf from Africa, India and Local (Indonesian) which Cultivated in Bojonegoro Indonesia

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ABSTRACT

Purpose: The research was to determine specific parameters (macroscopy and microscopy) and non-specific parameters (loss on drying, total ash content, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter), heavy metal contaminant (Pb, Hg, As, Cu) and microbial contaminant (Total Plate Count and Total Yeast Mold). Research also determined a total flavonoid of *M. oleifera* leaf.

Methodology: The method carried out according to the procedure stated in the *Materia Medika Indonesia* 5th edition. Result test compared to the standard stated in *Indonesian Herbal Pharmacopoeia* 2nd edition. Methods to detection Contaminants were referred to WHO guidelines for assessing the quality of herbal medicines. The determination of total flavonoid was performed by spectroscopic method.

Results: Crude drug of *M. oleifera* leaf meet the specific parameters (macroscopy and microscopy). Non-specific parameters of Africa, India, Local *M. oleifera* leaf shows that loss on drying (8.06 ± 0.03 ; 8.89 ± 0.31 ; 7.56 ± 0.17) %, total ash content (8.64 ± 0.43 ; 10.64 ± 0.90 ; 15.31 ± 0.87) %, acid insoluble ash content (0.56 ± 0.08 ; 0.35 ± 0.01 ; 0.36 ± 0.06)%, water-soluble extractive matter (21.38 ± 1.39 ; 30.12 ± 2.06 ; 12.68 ± 1.12) %, ethanol-soluble extractive matter (39.37 ± 1.51 ; 27.74 ± 2.44 ; 27.09 ± 1.43)%, contaminant test including heavy metal contaminant shows that Pb, Cd, As and Hg were not to be detected. Microbial contaminant (Total Plate Count and Total Yeast Mold) under limits of WHO standart. Total flavonoid content of Africa, India, Local *M. oleifera* leaf were 8.12 ± 0.52 mg/ 100 mg QE; 10.69 ± 0.15 mg/ 100 mg QE dan 13.08 ± 0.08 mg/ 100 mg QE respectively.

Conclusion: *Moringa* Leaves meet specific parameters (macroscopy and microscopy test). Non-specific parameter tests of Africa, India, Local *Moringa* Leaf shows that loss on drying, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, contaminant test including heavy metal contaminant shows that (Pb, Cd, As and Hg) and microbial contaminant (Total Plate Count and Total Yeast Mold) meet the standard required, only total ash content did not meet the standard. Total Flavonoid content shows that the extract of *M. oleifera* from local (Indonesia) variety is higher than others.

Key words: *M. oleifera* leaf, Standardization, Crude Drug

Applications/Originality/Value: Quality of Crude drug of *M. oleifera* leaf from Africa, India and Local (Indonesian) which Cultivated in Bojonegoro Indonesia. There was no study compare a different *M.oleifera* variety which cultivated at the same places. The findings could be helpful in the identification and authentication of *M. oleifera* in future for further research and utilization

INTRODUCTION

Moringa oleifera (Moringaceae) contains flavonoid compound such as apigenin, quercetin, ramnetin and camferin (Misra et al, 2012; Leone et al, 2015) and alkaloid moringinin (Mehra et al, 2017). *Moringa* leaf with various potential active pharmacology compound used as antioxidant, anti-ulcer, hyper cholesterol, anti-hypertension, anti-inflammatory, anti-tumour, anti-microbial and anti-diabetes (Asiedu-Gyekye et al, 2014; Ferreira et al, 2014). *M.oleifera* leaf also high nutritive content besides of the medicinal functions. The micronutrients content is 7 times the vitamin C of oranges, 4 times the vitamin A of carrots, 4 times the calcium of milk, 3 times the potassium of bananas, and protein in the two yogurts (Aminah et al, 2015). Widely use of

moringa leaf in the community encourages the important thing of standardization. Standardization of herbal drugs means confirmation of its identity, Quality and purity (Bijauliya et al, 2017). Standardization aims to ensure the quality of herbs used as raw material for herbal medicine product and compared with standard quality of crude drug in Indonesian Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017).

Standardisation parameter including specific parameters and non-specific parameters. Specific parameters of standardization including plant identity, organoleptic, macroscopy and microscopy, marker / active compound content (total flavonoid content, phenolic content, volatile oil, etc). Non-specific parameters including loss

on drying, total ash content, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, residual solvents, pesticide residues, heavy metal contaminant and microbial contaminant (DEPKES, 2000).

Quality control of herbal plant refers to WHO standard including pesticides residues, heavy metals contaminant and microbial contaminants. Contaminant was an undesired introduction of impurities of a chemical or microbiological nature, or foreign matter, into or onto a starting material, intermediate product or finished herbal product during production, sampling, packaging or repackaging, storage or transport (WHO, 2011).

WHO declared that heavy metal contaminant including Lead (Pb) and Cadmium (Cd). BPOM RI (National agency of drug and food Republic of Indonesia) declared that heavy metal contaminant Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg). Microbial contaminant in WHO regulatory, including total aerobic bacteria, total mould and yeast, enterobacteria, gram-negative bacteria, *Eschericia coli* and *Salmonella*. Furthermore, microbial contaminant based on BPOM RI (Indonesian Food and Drug Agency) including total plate count, total mould-yeast, aflatoxin and bacteria *Eschericia coli*, *Salmonella spp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* must be absent. Many factors, such as geographical location, climate, temperature, agricultural process, harvesting process, post-harvesting process (drying method, storage) influence the quality of herbal material and chemical content of them. The chemical content of the medicinal plant was determined by the species of medicinal plants to their varieties (Dewoto, 2007 ; de Freitas Araujo& Bauab, 2012).

Plant material

Crude drug of *M. oleifera* leaf was collected on March 2019 from Bogo village, Kapas, Bojonegoro District, Indonesia. The plant leaves collected from 3 different varieties: Africa, India, Local (Indonesia) which cultivated in Bogo. *M. oleifera* leaf was identified and authenticated by Forest Farmer Group (Kelompok Tani Hutan) Bojonegoro.

METHODS

Extraction

M. oleifera leaf was dried at room temperature and powdered with blender, then sifted with sieve 60 mesh. The sample was extracted by maceration method with ethanol 96% at room temperature for 3x24 h. The liquid extract was evaporated with a rotary evaporator.

Specific Parameter of Standardization

Specific parameters of standardization including the identity of the plant, organoleptic, macroscopy and microscopy, marker / active compound content (total flavonoid content, phenolic content, volatile oil). Macroscopy and microscopy of the *M. oleifera* leaves was studied according to the method in Materia Medica Indonesia (DEPKES RI, 1989) and Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017). Identification a powder of *M. Oleifera* leaf using microscopes binocular Olympus CX-23.

Non-Specific Parameter of Standardization

Non-specific parameters including loss on drying, total ash content, acid insoluble ash content, water-soluble extract content, ethanol-soluble content according to the method in Materia Medika Indonesia (DEPKES RI, 1989) and Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017). Methods for detection Heavy metal contaminant and microbial contaminant referred to WHO guidelines for assessing the quality of herbal medicines with references to contaminants and residues.

Determination of Total Flavonoid

Total flavonoid was analyzed using aluminium chloride colourimetric method according to Sulastri et al (2018). Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in ethanol 96% and diluted to 8, 10, 12, 14, 16 and 18 µg/mL. 1 ml of each concentration of standard solutions, as well as 1 ml of each sample solution, were mixed with 3 mL ethanol 96%, 0.2 mL of aluminium chloride 10%, 0.2 mL potassium acetate 1 M and 5.6 mL of distilled water. The mixture was incubated at room temperature for 10 min with intermittent shaking. The absorbance was measured at 376 nm using Shimadzu B UV-Vis spectrophotometer. Total flavonoid was calculated as mean ± SD (n = 3) and expressed as weight of quercetin equivalent (QE)/ 100 mg extract.

RESULTS

Specific Parameter of Standardization

Macroscopy

M. oleifera can be cultivated at low and high altitude. Macroscopy of crude drug *M.oleifera* leaves was green to brownish green, *cimcumcriptio* was *ovatus* or *obovatus*. Leaf length of 10 mm to 30 mm and width 4 mm to 10 mm. *Apex Folii* was *obtusus*, *Basis Folii* was *rotundatus* and *Margo Folii* was *integer*. Macroscopy of *M.oleifera* leaf shown in Figure 1 and Table 1.



Figure 1. Macroscopy Crude Drug *M.oleifera* Leaf

Table 1. Macroscopy of *M. oleifera* Leaf




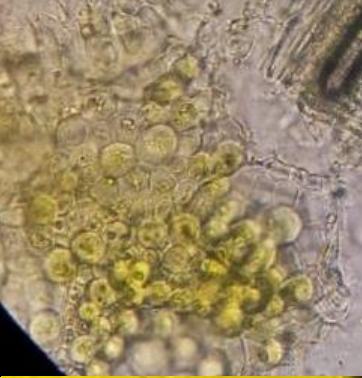


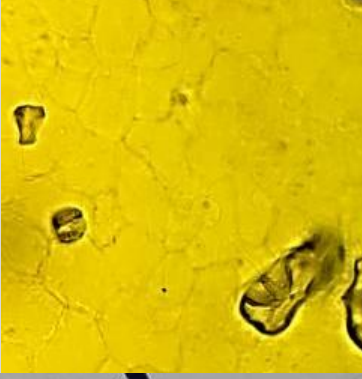

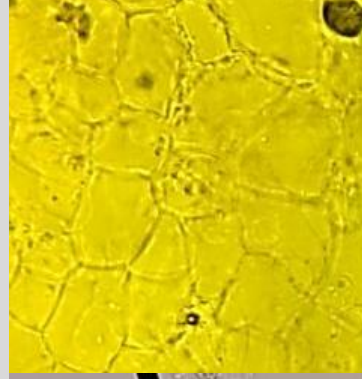



Parameters	Indonesia Herbal Pharmacopoeia 2 nd edition (DEPKES RI, 2017)	Materia Medica Indonesia 5 th edition (DEPKES RI, 1989)	Result		
			Africa	India	Local (Indonesian)
<i>circumscrip-tio</i>	<i>orbicularis or ovatus- oblongus</i>	<i>Ovatus or obovatus</i>	<i>ovatus- oblongus</i>	<i>Ovatus or obovatus</i>	<i>Ovatus or obovatus</i>
<i>Apex folii</i>	<i>Obtusus or rotundatus</i>	<i>obtusus</i>	<i>obtusus</i>	<i>rotundatus</i>	<i>rotundatus</i>
<i>Basis folii</i>	<i>acutus</i>	<i>rotundatus</i>	<i>rotundatus</i>	<i>rotundatus</i>	<i>rotundatus</i>
<i>Margo folii</i>	<i>integer</i>	<i>integer</i>	<i>integer</i>	<i>integer</i>	<i>integer</i>
<i>Nervatio Folium Compositum</i>	<i>penninervis pinnatus</i>	<i>penninervis imparipinnatus</i>	<i>penninervis imparipinnatus</i>	<i>penninervis imparipinnatus</i>	<i>penninervis imparipinnatus</i>
Colour	Green, yellowish green, brownish green	Green, brownish green	Dark green	green	green
Lenght	-	10 mm – 30 mm	15 mm – 25 mm	15 mm – 25 mm	15 mm – 2 mm
Width	-	4 mm – 10 mm	5 mm – 10 mm	10 mm – 13 mm	5 mm – 10 mm
Smell	odorless	specific	specific	specific	specific
Taste	-	-	Slightly bitter	Slightly bitter	Slightly bitter

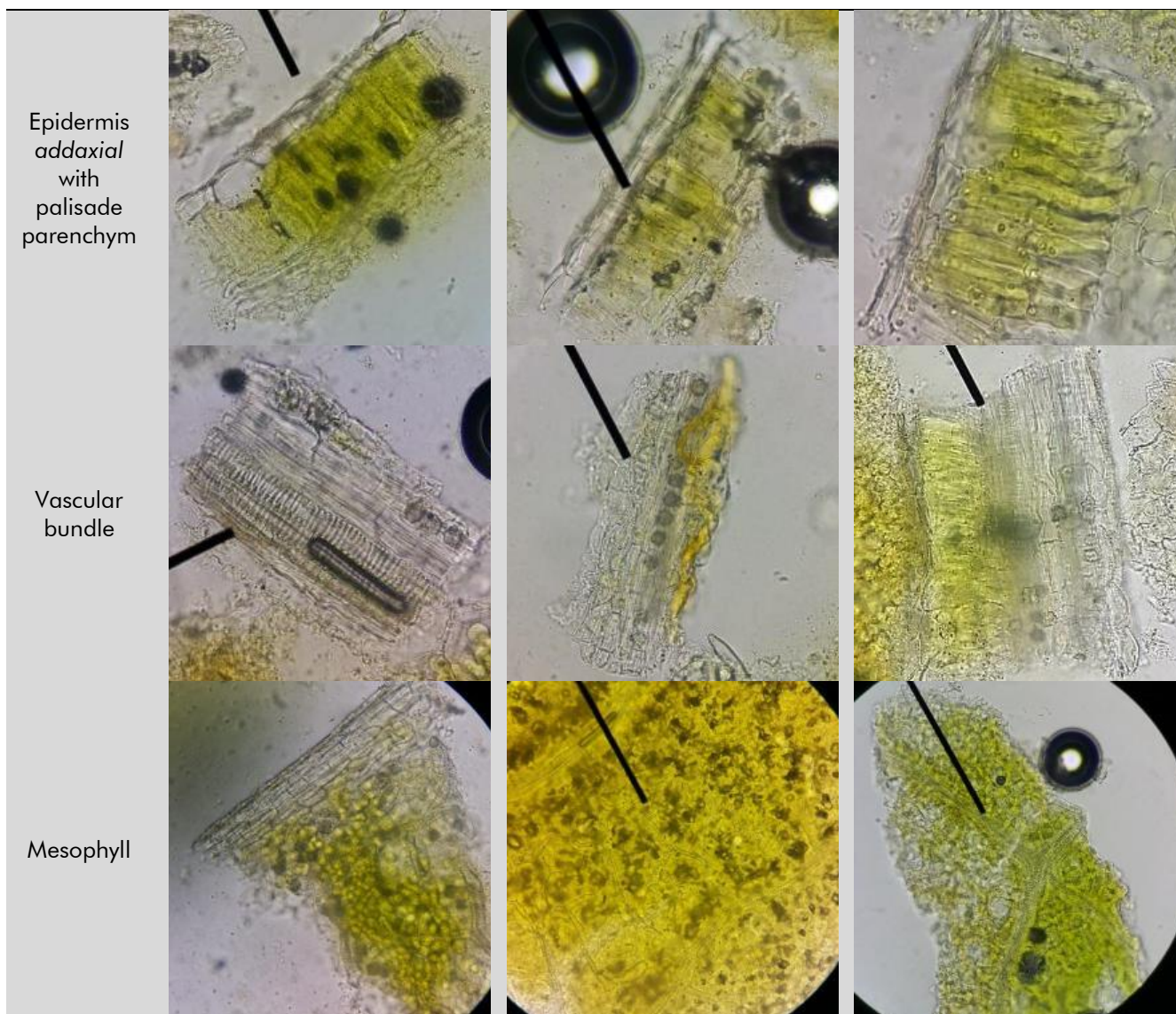
Microscopy

Microscopy of *M.oleifera* observed with an enlargement 400x of a binocular microscope. Identified or specific fragment of powder crude

drug *M.oleifera* leaf including trichomata, epidermis addaxial, xylem with spiral thickening and Calcium oxalate crystal. Table 2 shows microscopy of *M. oleifera*.

Table 2 Microscopy of *M. oleifera* Leaf

Parameters	Africa	Result India	Local (Indonesian)
Trichomata			
Pallisade parenchym			
Epidermis <i>abbaxial</i> with anomositic stomata			
Calcium oxalate Crystal			



Macroscopy and microscopy of crude drug *M. oleifera* leaf shows that Africa, India and local (Indonesian) varieties meet the criteria on *Materia Medica Indonesia* 5th edition (DEPKES RI, 1989) and *Indonesia Herbal Pharmacopoeia* 2nd edition (DEPKES RI, 2017).

Non-Specific Parameter of Standardization

Non-specific parameters including loss on drying, total ash content, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, heavy metal contaminant and microbial contaminant was analyzed. Table 3 shows the non-specific parameter of crude drug *M.oleifera* leaf.

Table 3. Non Specific Parameter of Crude Drug *M.oleifera* Leaves.

Parameters	Indonesia Herbal Pharmacopoeia 2 nd edition (DEPKES RI, 2017)	Materia Medica Indonesia 5 th edition (DEPKES RI, 1989)	WHO guideline s for assesing quality of herbal medicines (2007)	BPOM RI (Indonesian Food and Drug Agency) (2014)	Result		
					Africa	India	Local (Indonesia n)
Loss on drying (%)	< 10	-			8.06 ± 0.03	8.89 ± 0.31	7.56 ± 0.17
Total Ash	< 7.5	< 11			8.64 ±	10.64 ±	15.31 ±

Content (%)				0.43*	0.90*	0.87*
Acid Insoluble Ash Content (%)	< 0.9	< 1		0.56 ± 0.08	0.35 ± 0.01	0.36 ± 0.06
Water-soluble extractive matter (%)	> 4.9	> 5		21.38 ± 1.39	30.12 ± 2.06	12.68 ± 1.12
Ethanol-soluble extractive matter (%)	> 5.0	> 5		39.37 ± 1.51	27.74 ± 2.44	27.09 ± 1.43
Heavy Metal Contaminant (ppm)						
	Pb	≤ 10	≤ 10	Not detected**	Not detected**	Not detected**
	Cd	≤ 0.3	≤ 0.3	Not detected**	Not detected**	Not detected**
	As		≤ 5	Not detected**	Not detected**	Not detected**
	Hg		≤ 0.5	Not detected**	Not detected**	Not detected**
Total Plate Count (colony/ g)			≤ 10 ⁶	6.1 x 10 ³	1.8 x 10 ²	6.3 x 10 ⁴
Total Yeast Mold (colony/ g)			≤ 10 ⁴			
	Yeast			< 10	< 10	< 10
	Mold			4.0 x 10 ¹	< 10	3.8 x 10 ²

*Parameters did not meet the standard required

**Detection limit of Cd, Pb: 0.1 ppm, Detection limit of As, Hg: 0.001 ppm

Loss on drying was a measurement of the residual of a crude drug after drying which is counted as a percentage. Loss on drying aim to provide a maximum limit (range) a quantity of compound has been lost after the drying process (DEPKES RI, 2017). The result shows that loss on drying for *M.oleifera* leaf African, India, Local (Indonesia) was 8.06 ± 0.03 , 8.89 ± 0.31 , 7.56 ± 0.17 respectively. All the sample meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition. Strongly recommends effective drying to reduce moisture content to less than 10%. However, since the products easily attracts and reabsorbs moisture during milling and storage, contamination with moulds and other potential pathogens is a serious concern (Adu-Gyamfi & Mahami, 2014).

Total ash content aims to describe the internal and external material content of crude drug *M.oleifera* leaf. The material contaminant has been found since the harvesting process, time to harvesting, drying process until the methods using for extraction process. The sample of crude drug *M.oleifera* has been heated at high temperature (600^o C at the furnace) until al material becomes ash. All the organic compound will be reduced and evaporated, only an inorganic material has

been found. Total ash and acid-insoluble ash contents are important indices to illustrate the quality as well as purity of herbal medicine. Total ash includes "physiological ash", which is derived from the plant tissue itself, and "non-physiological ash", which is often from environmental contaminations such as sand and soil. Total ash content alone is not sufficient to reflect the quality of herbal medicines, since the plant materials often contain considerable levels of physiological ash, calcium oxalate in particular. Thus, the acid-insoluble ash content is another index to illustrate the quality of herbal (Rao & Xiang, 2009). The result shows that Total ash content for *M.oleifera* leaf African, India, Local (Indonesia) was 8.64 ± 0.43 , 10.64 ± 0.90 , 15.31 ± 0.87 respectively. all the sample did not meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition, and only Local (Indonesia) variety did not meet the required standard of Materia Medika Indonesia 5th edition (DEPKES RI, 1989). High mineral content and inorganic compounds such as calcium, oxalate, potassium, sodium, chloride and alkali can be found in the herbal plant material (WHO, 2011). The result shows that acid insoluble ash content for *M.oleifera* leaf African, India, Local (Indonesia) was 0.56 ± 0.08 , $0.35 \pm$

0.01, 0.36 ± 0.06 respectively. All the sample meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition and Materia Medika Indonesia 5th edition (DEPKES RI, 1989).

Water-soluble extractive matter and Ethanol-soluble extractive matter were described a quantity of active compound has been extracted after maceration 1x24 h with the selective solvent (water or ethanol). The result shows that Water-soluble extractive matter for *M.oleifera* leaf African, India, Local (Indonesia) was 21.38 ± 1.39 , 30.12 ± 2.06 , 12.68 ± 1.12 and for Ethanol-soluble extractive matter was 39.37 ± 1.51 , 27.74 ± 2.44 and 27.09 ± 1.43 respectively. All the sample meet the required standard of Indonesia Herbal Pharmacopoeia 2nd edition and Materia Medika Indonesia 5th edition (DEPKES RI, 1989).

Contamination of heavy metal must be limited because can induce a serious health problem in the human body. Crude drug as a raw material for herbal medicine must be meet the quality standard limitation of contaminant. Toxicity due to heavy metal such as Lead (Pb) can induce neurological disorders and kidney damage. Cadmium (Cd) can induce kidney damage, lung cancer and bones abnormality. Arsenic (As) can induce lung cancer and skin disorders, and mercury (Hg) cause neurological disorder and kidney damage (Shaban, Abdou & Y.Hasal, 2016). The result shows that contamination of heavy metal (Pb, Cd, As, Hg) for *M.oleifera* leaf African, India, Local (Indonesia) was not detected at the sample (*Detection limit of Cd, Pb: 0.1 ppm, Detection limit of As, Hg: 0.001 ppm*). Plants accumulate minerals essential for their growth from the environment and may accumulate some metals which have no known direct benefit to the plants. Herbal can be contaminated by these heavy metals via root uptake or by direct deposition of contaminants from the atmosphere onto plant surfaces. The study further suggested that *M. olifera* in polluted sites may accumulate trace metals in any of its parts. When the plant is to be considered for medicinal purpose and the need to regulate where the plants should be cultivated because of its ability to uptake trace metals (Agboola *et al*, 2016).

The herbal product is not permitted contains a microbial as a contaminant. Contamination of

microbial (bacteria or yeast) can cause a problem- related health such as diarrhoea, febris, and other condition that unwanted. Preparation on the herbal product must be a concern with hygiene and sanitary as in CPOTB (The good procedure to prepare herbal medicine) based on Indonesian regulatory or GMP (Good Manufacturing Product). The total number of microbial and fungi are limited, but microbe such as *E.coli*, *Salmonella spp*, and *P. aeruginosa* must be absent. The microbial contaminant can be present due to the preparation process did not refer to CPOTB or GMP. The results show that microbial contaminant (Total Plate Count (colony/g) and Total Yeast Mold (colony/ g) for *M.oleifera* leaf African, India, Local (Indonesia) was under the limit quality standard of BPOM RI (2014) (National agency of drug and food Republic of Indonesia). *M.oleifera* leaf powder when boiled (a rolling boil for 5 min) contains risk of contamination from *B. cereus*, *C. perfringens* type A, *Cronobacter*, enterohemorrhagic *E. coli*, *L. monocytogenes*, *Salmonella spp.*, and *S. aureus* is low to moderate, with only a moderate to serious risk posed by *C. perfringens* type C. However, when the is not boiled before consumption, the food safety risk is increased for all of the evaluated pathogens. Risk, can be mitigated when the powder is stored under the appropriate conditions to ensure there is no ingress of moisture and then processed in a hygienic manner to reduce contamination and/or cross-contamination by following hazard analysis critical control point or similar procedures (even in a home setting) including a heat treatment, i.e., boiling, to further reduce microbial hazards (Walia, Kapoor, & Farber, 2019).

Total Flavonoid Content

Flavonoid was an active compound in *M.oleifera* leaf, it was polar and soluble in water or ethanol (Sulastri *et al* (2018). Based on Indonesia Herbal Pharmacopoeia 2nd edition (DEPKES RI, 2017) flavonoid content of extract *M.oleifera* leaf not less than 6.3% as a quercetin equivalent. Determination of total flavonoid use quercetin as standards where the calibration curve equations obtained were $y = 0.025x + 0.111$ ($R^2 = 0.987$). The result of total flavonoid shows in Table 4. Total Flavonoid content showed that the extract of *M. oleifera* from Local (Indonesia) variety is higher than others.

Table 4 Total Flavonoid Content of *M. oleifera* leaf

Parameters	Indonesia Herbal Pharmacopoeia edition 2 (DEPKES RI, 2017)	Result		
		Africa	India	Local (Indonesia)
Total Flavonoid Content	6.3 % QE	8.12 ± 0.52 mg/ 100 mg QE	10.69 ± 0.15 mg/ 100 mg QE	13.08 ± 0.08 mg/ 100 mg QE

CONCLUSION

Moringa Leaves meet specific parameters (macroscopy and microscopy test). Non-specific parameter tests of Africa, India, Local Moringa Leaf shows that loss on drying, acid insoluble ash content, water-soluble extractive matter, ethanol-soluble extractive matter, contaminant test including heavy metal contaminant shows that (Pb, Cd, As and Hg) and microbial contaminant (Total Plate Count and Total Yeast Mold) meet the standard required, only total ash content did not meet the standard. Total Flavonoid content show that the extract of *M. oleifera* from local (Indonesia) variety is higher than others.

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TABLE OF CONTENTS

- [2021 - Volume 13](#)
- [2020 - Volume 12](#)
- [2019 - Volume 11](#)
- [2018 - Volume 10](#)
- [2017 - Volume 9](#)
- [2016 - Volume 8](#)
- [2015 - Volume 7](#)
- [2014 - Volume 6](#)
- [2013 - Volume 5](#)
- [2012 - Volume 4](#)
- [2011 - Volume 3](#)
- [2010 - Volume 2](#)
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SUPPLEMENTRY ISSUES

- [Volume 11](#)
- [Volume 12](#)

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[Instruction to Authors](#)

[Current Issue](#)

[Article In Press](#)

[Table Of Contents](#)

CURRENT ISSUE

volume 14, issue 1, Jan - Mar, 2022

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[Current Issue](#)
[Article In Press](#)
[Table Of Contents](#)

CURRENT ISSUE

volume 14, issue 1, Jan - Mar, 2022

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IJPR 9[3] JULY - SEPTEMBER 2017 SPECIAL ISSUE

July - September 9[3] 2017

[Click to download](#)

Volume 12, Issue 1, Jan - Mar, 2020

REVIEW

PHARMACOLOGICAL AND PHYTOCHEMICAL STUDIES OF SESBANIA

GRANDIFLORA (LINN) - A REVIEW

BC SEMWAL, S PANDEY, S SHARMA

Medication adherence using self-report measures among chronic disease patients: A review

SUSI KRISTINA,, GALIH PUTRI WULANDARI, APT

Diabetic Nephropathy: A Review of Pathophysiological mechanisms, Therapeutic interventions and Biomarkers

REEMA MITRA, PRADEEP GOYAL

Study of the Outcome of Bubble CPAP Ventilation in Neonates with Respiratory Distress

DR. S., DR. J. M. PAWAR

Features of the course of discirculatory encephalopatia in young people (a literature review)

URAZALIEVA D.A., INOYATOVA S.O., ABDULLAEVA N.N., KHILOYATOVA D.N., MADJIDOVA Y.N.

Hair follicle stem cells for vitiligo treatment: review

ULUGBEK SABIROV, ILONA NAZAROVA, FOTIMA AZIMOVA, FIRAS AL-NIAIMI, BOBUR TOIROV

Review Article on Assess the Non-Pharmacological Management for Knee Osteoarthritis

R. RAMANI, DR.V. HEMAVATHY

A Review on Role of Robots in Healthcare Setting: An Emerging Change in Healthcare Service

HIRANYA.S , LAVANYA PRATHAP, V. VISHNU PRIYA, PREETHA S

Chemotherapy and Radiotherapy Related Complications in Oral Cancer Patients - A Review

S. KAMALA, D. EZHILARASAN , A. S. SMILINE GIRIJA

Temporomandibular Joint Arthrocentesis - An Institution Based Retrospective Study.

TRINAINA SOMAS, MADHULAXMI MARIMUTHU, ARVINA RAJASEKAR

Mechanism of Multi Drug Resistance and Chemotherapy -A Review

PADMA PRIYAA,, DR. RAGHU NANDHAKUMAR, DR. SMILINE GIRIJA AS

Role of Natural Killer Cells in Innate Immunity - A Review

S. BALAMITHRA, RAGHU NANDHAKUMAR, SMILINE GIRIJA AS

Antibiotics as Root Canal Irrigants - A Review

S. UMayAL, ANJANEYULU K, A.S. SMILINE GIRIJA

Prevalence And Pattern Of Edentulous Space According To ACP Classification In Patients Undergoing Full Mouth Rehabilitation Treatment - An Institution Based Retrospective Study

DR.HARINI SRI, DR. NABEEL AHMED, DR. KEERTHI SASANKA

Pattern of Mandibular Third Molar Impaction- A Retrospective Study

REVATHI.B , GIRINA JAYARAJ, KIRAN KUMAR PANDURANGAN

Treatment Effects Of Twin Force Bite Corrector - Review

V.BALAJI , REMMIYA MARY VARGHESE

Influence Of Parenting Styles On Child Behaviour And Anxiety In A Dental Setup- A Cross Sectional Study

PAVITHIRAA SANKAR, DEEPA GURUNATHAN

Esthetic Crowns In Pediatric Dentistry- A Review

CIBIKKARTHIK T, DR.JESSY P

Clinical Application Of Mineral Trioxide Aggregate In Pediatric Dentistry - A Systematic Review



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NURUL AMANI, GEO MANI

Influence Of Various Cavity Disinfectants On Bond Strength Of Self Etch Adhesives- An Invitro Study

KEERTHANA T, SINDHU RAMESH

Decision tree analysis for personalised management of high and low risk oral leukoplakia.

UMAMAHESWARI.T.N , MUKUNDHCHAITHANYAV, MANJARI CHAUDHARY

Prevalence Of Dental Caries Among School Going Children Based On Icdas- ii

SHALINI SATHIYAMOORTHY, MEBIN GEORGE MATHEW

Clinical practice guidelines in the management of open apex cases

SHREE PANDEY, MANISH RANJAN

Prevalence of Defective Restorations and Factors Associated with Re-Intervention in Primary Teeth: A Retrospective Study

IVAN OBADIAH, EMG SUBRAMANIAN

Comparative Evaluation of Reduction of Microbial Count with And Without Irrigant Activation - An In Vitro Study

KRISHNAKANTH JAJU, IFFAT NASIM

Prevalence of Early Childhood Caries Among Patients Visiting A Private Dental Hospital

SARVESH J, MAHESH RAMAKRISHNAN, MURUGAIYAN ARUN

Tissue Engineering in Oral and Maxillofacial Surgery - A Literature Review

KALYANI.P , M.P.SANTHOSH KUMAR

Nutrigenomics In Periodontics - A Review

SUHAS MANOHARAN, NASHRA KAREEM

Augmented Reality: A Current Breakthrough In Dentistry - A Review

MULUMOODI SOWMYA, AJITHA.P, S.PRADEEP

Assessment of dental caries experience among corporate employees in Chennai - Cross sectional study

MAHESHWARI ELUMALAI, I.MEIGNANA ARUMUGHAM, R.PRADEEP KUMAR, D.SRI SAKTHI

Evaluation of Anti-bacterial Efficacy of Medicinal Plant Extracts and 0.2% Chlorhexidine against Oral Bacteria

S.VYSHIALI , D.SRI SAKTHI, I.MEIGNANA ARUMUGHAM, R.PRADEEP KUMAR

Comparing the Levels of Some Trace Elements with Liver Enzymes in Chronic Renal Failure Patients with Viral Hepatitis B and or C

ALAA HUSSAIN HASSAN, SUHAIR SHATTI SALIH, ALIA KATHEM SALIM

Infraclavicular Brachial Plexus Block For Upper Limb Surgeries In Adult Patients; Non-Systematic Review

AMJED QASIM MOHAMMED, MAJID FAKHIR ALHAMAIDAH, HUSSEIN ALI HUSSEIN, HUSSEIN ALKHFAGI, SAMI RAHEEM HASAN, ALI B. ROOMI

Role of Polymer Type and Ratio in Improving the Solubility of Voriconazole Solid Dispersion: A Comparative Study

SAJAD MONEIM HAKEEM, ATHMAR DAHIR HABEEB, GAITH ALI JASIM

A Morphometric Study of Pterion for Neurosurgical Procedures.

SARVASRI.T , S.SANGEETHA

Comparison of efficacy of Amitriptyline and Duloxetine sodium in reduction of pain in Temporomandibular joint disorder (TMD) patients- A Systematic Review

DR.YANDETI SRINIVASULU, PROF.DR.ABDUL WAHAB, DR.SENTHIL MURUGAN.P

Role of Prolotherapy In the Management of Temporomandibular Disorders -A Review

BALAJI V, DIVYA T, SREEDEVI DHARMAN

A Review on Prescription and Non-Prescription Appetite Suppressants and Evidence Based Method to Treat Overweight and Obesity

, JAVAD NAZAFAT FERIZI, AHMAD ASHOURI, MOHAMMAD EBRAHIM HOKMABADI, SAJEDEH SABEGHI, ROZA SALEHIAN

Current Approach In The Development And Validation Of Bioanalytical Method.

, KHEDKAR AMOL NAVNATH, S.SATHESH KUMAR

RESEARCH

Evaluation of the Effects of Valeriana of Ficalinis Hydroalcoholic Extract on the Morphology of Cerebral Hippocampus Astrocytes in Rats

*ASGHAR HEIDARIAN, HAMDOLLAH DELAVIOZ, AMROLLAH ROOZBEHI**

Comparison of Common Antibiotic Therapies with Agicoat Nano Crystalline Dressings on Non-Infected Wounds

MOHSEN AVAZPOUR, REZA AZIZKHANI, MEHRZAD BARGHI KAR

Investigating the Factors Affecting Cesarean Section in Fasa: a Case-control Study

HALEH GHAEM1, SEYEDEH LEILA DEGHANI, MASOUMEH MOUSAVI

An Investigation Of The Effect Of Positive Thinking Training And Pray On Depression In Elderly Women Referring To Imam Reza Specialty And Subspecialty Clinic In Shiraz, Iran

FARIBA (PHD), MAHTAB NARAGHI RAD (MS)

The Comparison of Bacterial Colonization in the Centralized Venous Catheters by Peripheral Vessel in the Upper Limbs with the Lower Limbers of the Hospitalized



01174879

Premature Newborns in the Neonatal Intensive Care Unit*ROYA SHARIFI, SEDIGHEH MONTASERI, MITRA EDRAKI***Coping Styles with Stress in Nursing and Midwifery Students***FATEMEH MAHMOUDI, SHEKOOFE HAMZHIKIA, ZANDIRAAD PARVIN***Post Intensive Care Unit mortality and complications relative frequency in adulthood patients discharged in educational Hospital of Yasuj from October 2017 to March 2018***AMIN MOTLAGH, NASTARAN FOOLADI, JANMOHAMAD MALEKZADEH***Oxidative Stress And Endothelium Disfunction In Athletes As A Risk Factor For Developing Cardiomyopathy Of Overstrain***V.S. VASILENKO, Z.V. LOPATIN, V.V. OREL , A.K.KHUSHTOVA***Synthesis of New Thiocarbamates and Evaluation of their Cholinesterase Inhibitory Effect***BAHAREH VAYEGHAN***A Guide to Medical Teachers for Analysis Distractors Options***HABIBOLAH REZAEI, NASRIN DEGHANI***Development And Preclinical Evaluation Of Preparation Effectiveness For The Treatment Of Hard Tooth Tissues After Temporary Filling Removal***VITA B?R?ZENTSEVA, ELENA A KUZMINA, LYDMILA L. GAPOCHKINA, SERGEY V. POKLAD, TATYANA N. SHINKARENKO, ALEXANDER A. KOPYTOV***Minimally Invasive Methods Of Treating Complications Of Cholelithiasis***VLADIMIR KULIKOVSKY1, ALEXANDER A. KARPACHEV, ALEXANDER V. SOLOSHENKO, ANDREI L. IAROSH, VICTOR K. GOSTISHEV, ALEXANDER F. CHERNOUSOV, ANJELA V. GNASHKO***ETHICS OF METHODOLOGY AND METHODS OF RESEARCH OF FAMILY AND DEMOGRAPHIC PROBLEMS***LEBEDEVA VLADIMIROVNA, KABANOV OLEG VLADIMIROVICH***Problems Of Dispensing Prescription Drugs In Pharmacies***IRINA FILINA1, ANGELINA G. KHVOROSTYANOVA, IRINA V. SPICHAK, GALINA V. VARENYKH***Isolation, characterization and identification of contaminant bacteria from sugarcane (Saccharum officinarum L.) in vitro culture***T. MUNAWAR, YISEHAK TSEGAYE REDDA , T. TSEHAYEKIDUS, ZENEBE TEKA***Comparative Evaluation Of Antimicrobial Effect Of Turmeric Mouthwash And Chlorhexidine Mouthwash Against Oral Microbes In Orthodontic Patients- An In-Vitro Study***DR.ARATHI M, DR.S.P.SARAVANA DINESH, DR.A.S.SMLINEGIRIJA***Bosentan loaded Nano structured lipid carriers: A novel formulation and evaluation of their In-vitro, Ex-vivo and In-vivo characteristic using 3 full factorial design.***DR. PRAJAPATI, MR. UMANG VARIA***Assess the impact of post traumatic stress disorder in road traffic accident survivors in Erode district, Tamil nadu***ARUL PRAKASAM, ANN MARY JOSEPH, NANISHA ZACHARIAH, SHARON JOY***AN OVERVIEW ON RECENT ADVANCES IN POLYMERIC NANOPARTICLES AS A COLLOIDAL DRUG CARRIER***ABHIJIT CHOUDHURY, LALDINCHHANA***Synthesis, molecular docking, cytotoxicity and antioxidant activity evaluation of 4-(3- chloro-1, 4-dioxo-1, 4-dihydronaphthalen-2ylamino) benzenesulfonamide derivatives***PALANICHAMY KUMAR, KURUBA BHARATH KUMAR, ASIR OBADIAH, RAMAN MOHANAPRIYA , ARULAPPAN DURAIRAJ , SUBRAMANIAN RAMANATHAN , SAMUEL VASANTHKUMAR***A Framework base analyzing cancer cells in blood Image segmentation by using convolution neural networks***V. KAKULAPATI, S. MAHENDER REDDY***Characterization of Nutraceutical Properties of Date Palm (Phoenix dactyliferaL.) Kernels***HOSNI. S, ABD GANI. S.S , HALMI. M.I.E, ZAIDAN. U.H***"A Comparative Study On Ruksha And Snigdha Virechana In The Management Of Amavata (Rheumatoid Arthritis)"** *MAHESH P, BM SUSHMA, RAVI SAHU, IMLIKUMBA, K B ROY, THAKKER N***ANTI HYPERGLYCEMIC EFFECT OF OCIMUM SANCTUM, TRIGONELLA FOENUM GRAECUM, CURCUMA LONGA BY ORAL GLUCOSE TOLERANCE AND ORAL STARCH TOLERANCE STUDY IN ALBINO RATS***M.MUNIAPPAN***POTENT a-AMYLASE INHIBITION ACTIVITY OF NATURAL GUMS: AN IN-VITRO ANTI-DIABETIC STUDY***PRASHANT SINGH, RITU M GILHOTRA***Microwave-assisted synthesis of ZnO nanoparticles and its photocatalytic activity in degradation of Rhodamine B dye***HASSAN HABEEB, ABEER A.JAFFER***Characters anatomy of CorchorusolitoriusL. from Malvaceae family cultivated in Iraq***MUAZAZ AL-HADEETHI, JWAD K. ALI , ZAINALABDEEN AL-MOUSSAWI*

The therapeutic effect of the active compound derived from green tea on induced infection of Entamoebahistoltyca in mice

SURA ABDULATEEF, HASANA. AAL OWAIF , MANHAL F. AHMED

Spectrophotometric Method for Determination of Ranitidine hydrochloride in Pharmaceutical Formulations using Cobalt Thiocyanate as Chromogenic Reagent after Cloud Point Extraction

AZHAR GHALI

Synthesis, characterization and bioactivity study of mixed-lig and complexes with some metal ions for new dithiocarbamate,3-aminophenol

LEKAA HADI, AHMED T. NUMAN

Preparation, spectral characterization and biological activity of somenewbimetalic complexes derived from{ 2,2' ((5-methyl-1,3-phenylene)-bis- (oxy))-bis-N' (E')-2-hydroxybenzylideneacetohydrazide}ligand

MAHA MUSTAFA, RIYADH M. AHMED

Synthesis and Antioxidant Study of new1,3Oxazepin-4,7-dione and1,2,3-Triazole derivatives

HAWRAA MAZYED, RIYADH J. NAHI

Synthesis of Some New Schiff Bases Starting from 2,2 DimorpholinyI Acetic Acid and Evaluating the Biological activity

SALEEM S., EMAN M. HUSSAIN

Activity of I gA Protease secretion by Proteus vulgaris From Urinary Tract Infection Patients

HANAN SUBHI, FATIMA RAMMADAN ABDUL

Antibacterial evaluation of some new benzimidazole derivatives

MUHANNED MAHMOUD, SURA K. IBRAHIM, MUAYAD AHMED RDAIAAN

Biological synthesis of copper oxide nanoparticles using spinach extract

SAFA SHNAIN, A. HABEEBALSHAMSI

Evaluation of anticancer potential for L-glutaminase purified from Bacillus subtilis

ASMAA HUSSEIN, SHYMAA I. ALBARAZANCHI , AHMED F.AL-SHANON

Assessment of Serum Interleukin-6 and Leptin Levels in Postmenopausal Iraqi Women

MAHDI H.HUMMADI, HIBA S.AHMED, ZAINABS.HUSSEIN, ZINAHS.ALI

Comparison of efficacy of rosuvastatin as cholesterol synthesis inhibitor with atorvastatin and simvastatin in patients with hypercholesterolemia

BUSHRAMAHMOOD HUSSEIN, SALIH MAHDI SALMAN, ZUHAIRMAROOF HUSSEIN

Features of sexual development, state of the pituarygonad system and measures of secondary prevention in sick children with chronic bronchitis

BOBOMURATOV AKRAMOVICH, SHARIPOVA OLIYA ASKAROVNA, MAMATKULOVA DILRABO HAMIDOVNA, BAKHRONOV SHERZOD SAMIYEVICH

Bioanalytical Method Development and Validation For Simultaneous Estimation of

Vildagliptin & Enalapril Maleate In Rabbit Plasma (K2 EDTA) Using Rp-HPLC

B.KISHORE REDDY, KOTHAPALLI BONNOTH CHANDRA SEKHAR, KRISHNA MOHAN CHINNALA

Effect of Sodium Hyaluronate and Gentle Curettage in Relief the Pain of Dry Socket

Synthesis and characterization of some heterocyclic compounds derivative of some chalconeswith Evaluation for Antibacterial Activity for Some of them.

DHAMER. M, AYAD. SULAIMAN., IBTISAM .KHALIFA.

Structural And Biological Study ofF New Schiff Base Derived From 2- Amino-6methoxybenzothaizole With Isatin And Its Mixed Complexes With Curcumin For Some Metallic Ions

WIDYAN .F.HASSAN, SAJID M. LATEEF

Spectrophotometric Determination of Candesartan Cilexetil and Atenolol In Pure and Pharmaceutical Forms

SURA L.ALKHAFAJI, RAJWAN A. ALAZAWY, ABDULBARI MAHDI MAHOOD

Activity of IgA Protease secretion by Proteus vulgaris From Urinary Tract Infection Patients

HANAN SUBHI, FATIMA RAMMADAN ABDUL

Study effect of sulfur containing amino acid supplementation on oxidative stress, inflammatory markers and serum lipid in relation to obese subjects

FALAH SHARI, ANWAR YONIS IBRAHIM, JUBRAN K. HASSAN, H. N. K. AL-SALMAN

Drug Utilization in HF Patients: Exploring Reasons to Improve Adherence at Tertiary Care Unit

KAVITA BANSAL, SATYENDRA KUMAR RAJPUT, VINOD SHARMA, V. S. MATHUR

Features of sexual development, state of the pituarygonad system and measures of secondary prevention in sick children with chronic bronchitis

BOBOMURATOV AKRAMOVICH, SHARIPOVA OLIYA ASKAROVNA, MAMATKULOVA DILRABO HAMIDOVNA, BAKHRONOV SHERZOD SAMIYEVICH

Crystal Growth and Structural Refinement of Chlorzoxazone (5-Chloro -3H-benzooxazol-2 2-one): An Effective Muscle Relaxant

RANJANA SHARMA, DIXIT PRASHER DIXIT PRASHER, R. K. TIWARI

Assessment of the Parameters of Malnutrition Among Internally Displaced Children in Najaf Province

FURQANHASHIM HUSSEIN, MOHAMMED D. AL-REKABI, NOOR HASSOON ALQARAGHULI

- Anti-Hypercholesterolemic Activity of Ethnolic Extract of Gomphrena globosa Flowers and its Phytochemical Screening**
AULIA RAHMAN, TANTI AZIZAH SUNJONO
- Anemia Prevalence in Patients with Myocardial Infarction Referring to Imam Sajjad Hospital of Yasuj City during 2017**
AKRAM SHAHAMAT, JANMOHAMAD MALEKZADEH, MARYAM SHAHAMAT
- Comparison of the Effect of Competence Stimulus in Two Methods of Spaced Learning and Flexible Fridays on the Nursing Students Critical Thinking**
OSSEIN HEJR, PARISA ZAJ, NAEEM ABDI, MOHAMMADSAEED MIRZAEI, MOHAMMAD BEHNAMMOGHADAM, BAHRAM ARMOON, MORTEZA HEMMAT
- Examination of Life Quality, Anxiety and Social Support in Adult Patients with Asthma and Chronic Obstructive Pulmonary Disease (COPD) admitted to Yasuj Pulmonary Clinic in 2017**
MOHAMMAD GHOLAMNEZHAD, MOHAMMAD MALEKZADEH, SAJAD HASSANZADEH
- Correlation between ABO Groups with Viral Load in Chronic Hepatitis B Infection**
MALIHEH METANAT, ALIREZA ANSARI-MOGHADAM, SAEED JAVDANSIRAT
- The use of Complementary Medicine in Pregnancy Nausea**
TABATABAEI MARZIEH, ASLANI MARZIEH, SAFARI MEHRI, PARVINSADAT ESLAMNIK
- Asthma Knowledge and Level of Education Predict Medication Adherence in Patients with Asthma**
ZAKKY CHOLISOH, YULITA KRISTIANA, LARAS PERMATASARI
- Formulation and Antibacterial Activity of Ceremai (Phyllanthus acidus) Lozenges Using Three Different Binders**
SETYO NURWAINI, RATNA YULIANI, WARDATUL JANNAH, AWALIA SINAKA, DEVI UTAMI YULISTYANTI
- Hermit's Water Lily (Limncharis Flava) Extract: A Potential Anti-Inflammatory Agent with Minimal Ulcerogenic Toxicity on Stomach**
TANTI SUJONO, GALANG ADI WIBOWO, ZAKKY CHOLISOH
- Investigation of Immunomodulatory Active Compounds from Faloak (Sterculia quadrifida R.Br.) Bark Fractions**
MUNAWAROH, R., SISWADI, SETYOWATI, E.P., MURWANTI, R., HERTIANI, T.
- The Sinergistic Effect of Black Rice Bran Extract and Glibenclamide on Protecting Renal, Hepatic, and Pancreatic Cells in Alloxan Induced Rats**
ARIFAH WAHYUNI, LUKMAN HAKIM, ARIEF NURROCHMAD, PUJI ASTUTI
- Study Preclinical Of Methanol Extract Of Melinjo (Gnetum gnemon, L.) Peel In Hyperuricemia Rat Model**
ARIFAH WAHYUNI, MUHAMMAD REZA NAJMI, AHMAD FAUZI
- Prayer Therapy in Islamic Teachings**
MOHAMMADJAVAD FARAHBAKHS, ABBAS ALI EBRAHIMMEHR, HAMIDREZA SOHRABI
- A Comparison of the Effect local Injection of Bupivacaine on the Post Tonsillectomy Pain in Children**
GHOLAMABBAS SABZ, AKVAN PAYMARD, ARDASHIR AFRASIABIFAR, ROGHAYEH PANNAHI, MAHIN ROOZITALAB
- Long-Term Outcomes of Patients with Periodontitis Submitted to Periodontal Therapy and Implant Placement: A Systematic Review and Meta-Analysis**
SEYED MONAJEM, FARZAD SARANDI, MEHRAB TAKHTDAR
- Surgical Outcome of the Application of Expandable Endoprosthesis in Children: A Systematic Review and Meta-Analysis**
REYHANEH RAHIMI, ALI MOHAMMAD ABDORAHIM KASHA, MEHRAB TAKHTDAR
- Prevalence and Risk Factors of HBV, HCV, and HIV Infection in Thalassemia Patients Requiring Oral and Maxillofacial Care in an Iranian Sub-population**
EHSAN ALIABADI, SAEID TAVANAFAR
- Self-Reported Quality of Life after Maxillofacial Surgical Treatment: A Prospective Cohort Study** ✨
EHSAN ALIABADI, SAEID TAVANAFAR
- Root Canal Variations of Maxillary First Molars: A Case Series** ✨
YASAMIN GHASHRAMANI, ALI POOLADI
- Compare Cement- And Screw-Retained Retention Systems in Fixed Implant-Supported Restorations: A Systematic Review and Meta-Analysis**
SARA KHALEGHI, MARYAM FIROUZMANDI, AZADEH KIANIPOUR, AZADE FARHANGNIA
- valuate the Difference in Failure Rates between Amalgam and Composite Resin Posterior Restorations: A Systematic Review and Meta-Analysis** ✨
MALIHE KARRABI, FARZAD SARANDI, AZADEH KIANIPOUR
- valuation of the Occupational Health Status of the Yasuj Cement Factory Personnel in 2017** ✨
SEYED CHERAGHZADEH, AFSHIN MANSOURIAN, SAJAD HASSANZADEH
- Comparison of Endovascular Aneurysm Repair (Evar) with Open Surgical Repair in Elective Patients with Abdominal Aortic Aneurysm Performed by Vascular Surgeons: OneYear Outcome** ✨
JAVAD SALIMI, HOSSEIN ZABIHI MAHMOUDABADI, MD, AMIR ASHRAF-GANJOUEI, MD, PEZHMANN FARSHIDMEHR, MD, REZA HAJEBI, MD, EHSAN RAHIMPOUR, MD, KHOSRO NAJARI, MD, MORTEZA TAGHAVI, MD,

MOHAMMAD REZA ZAFARGHANDI, MD , SEYYED MOHAMMAD SADEGH VAZIRI, MD

Anti-elastase of Leaves and Stem Bark Extract of *Garcinia daedalthera* Pierre

ENENG SILFI, BERNA ELYA , YESI DESMIATY, HANITA OMAR



Antihyperglycemic and anti-oxidative potential of the combination of *Channa striata*, *Zingiber zerumbet* and *Nephelium lappaceum* extracts in alloxan-induced diabetic mice

OCTAVIANA KURNIA, FIRDAUS FIKRI DZAKI BAKRI , MUHTADI, ERINDYAH RETNO WIKANTYASNING , ANDI SUHENDI, EM. SUTRISNA



Antiinflammation Effect of *Tabat Barito* (*Ficus deltoidea*) Leaf Extract on Small Intestine of Mice Induced by Dextran Sodium Sulfate

KUSMARDI KUSMARDI, RIZKY DINI FITRIYASA



In Vitro Phytochemicals, Antioxidant and AntiAcetylcholinesterase Activities of *Ficus Deltoidea* Infusion

NOR MOHD, SOHAIL LATIF, SAMEERA ABBAS, IHSAN GHAZALI, FARIDAH KORMIN



A Study on Antimycobacterial Activity and Phytochemical Constituents of *Dipterocarpus sublamellatus* foxw. Hexane Extract (DSHE)

SITI SABRAN, MUHAMAD HARITH MAZLUN, ZUNOLIZA ABADULLAH, MARYATI MOHAMED, MOHD. FADZELLY ABU BAKAR, FURZANI PA'EE, ALONA CUEVAS LINATOC

Standardization of a Crude Drug *Moringa oleifera* Leaf from Africa, India and Local (Indonesian) which Cultivated in Bojonegoro Indonesia

NIKMATUL EKA, KARTINI, LINTANG KARINA PUTRI



Effects of topical Phenytoin, Chitosan, Dextrin, and Chitosan-Dextrin Combinations in Experimentally induced Thermal Injury in Rabbits

HMED ABD, ABDULKAREEM H. ABD, MUHAMMED A. H. ALDABAGH



Cerebral palsy with mental retardation: A case report

NORAH VANLALHRIATMAWII, LALRAMENGMAWII, REGIL VARGHESE, TENZIN TSUNDUE, KHAYATI MOUDGIL*

A COMPARATIVE STUDY OF EPIDURAL BUTORPHANOL AND TRAMADOL FOR POSTOPERATIVE ANALGESIA IN ORTHOPEDIC LOWER LIMB SURGERIES

, KIRAN A V, SUNIL B. VASUDEVARAO, RAGHAVENDRA B GOUDER

RP – HPLC Developed Method for paracetamol, Caffeine and Chlorpheniramine Maleate Estimation in Pure and pharmaceutical formulation

IQBAL MOHAMMED, DUAA A. YASS, INAM H. KUDHAIR



Isolation and Diagnosis of Fungal Isolates Producing Antimicrobial Agents from Petroleum Soil

ALAA HUSSEIN



Synthesis of Hyper Branched Polyester Polymers Containing Thiobarbituric Acid and Study of its Optical Properties

SHIREEN RASOOL, ALI S. ALLW, ALI JASSIM AL-ZUHAIRI, RAWAA HEFDHI



Synthesis and biological evaluation of Schiff bases and pyrazole derivatives derive from chalcones, (2E)-1-(4-aminophenyl)-3-(2-furyl)prop-2-en-1-one

ALI HASSAN, HAMID H. MOHAMMED



Spectrophotometric Determination of Olanzapine by Charge-Transfer Complex Formation in its Pure Form and Pharmaceutical Formulations

HAMSA YASEEN, RUAA M. MAHMOOD, ROKAYIA S. SADEQ



Synthesis and Studies the Microbiology Activity for Some Thiophene Derivatives

HAMID MOHAMMED, ZAIZAFUONE N.NASIF, ZAINAB N. MAGEED



The Association of Cluster of Differentiation 34 Gene (CD34) Polymorphism with Oral Ulceration in Systemic Lupus Erythematosus Iraqi Patients

MAYTHAM ALI, TAGHREED F. ZAIDAN



Epidemiologic Evaluation of Hemodialysis Patients Referred to Dialysis Ward of Yasuj Shahid Beheshti Hospital in 2018

SAEED JAVDANSIRAT, AKVAN PAYMARD, SAADAT MEHRABI



Evaluation of Submandibular Gland Fossa Variety by CBCT in Patient Referring to Oral and Maxillofacial Radiology Center in Yasuj in the Academic Year of 2017-2018

FATEMEH SADEGHI, SABAH MIRHADI , JANMOHAMAD MALEKZADEH, SEYED ALISALEH GHOLAMI



A Psychoanalytic Health Survey on the Iranian EFL Learners' Emotional Intelligence and Their Language Learning Approach

SEYEDEH TABATABAEI, MOHAMMAD RASOUL HOMAYOUN, SEYED MOHAMMAD ALI MANSOORIAN



Neurogenic Pulmonary Edema Post Head Trauma: A Case Report

MOHAMAD SISAKHT, AKVAN PAYMARD, SAEED JAVDANSIRAT



Effect of Two Types of Surface Coatings of Dental Implants on Implant Support for Bone and Tissue around Prostheses a Systematic Review and MetaAnalysis

MEHDI ZARE, BENTOLHODA KORDAFSHARI , ARMIN KHOSRAVI



Proportion of Maxillofacial Trauma Resulting among Children: Systematic Review and Meta-Analysis



- LOTFOLLAH HAKIM, FARNAZ DEHABADI, MOHAMMAD AMIR ALIZADE TABRIZI, SHABNAM GARJANI*
- ontents of Pedagogical Technology of Development of Coordinating Abilities in Hand-Held Fighters** ✱
LARISA KADUTSKAYA, TATIANA A. MIRONOVA, EKATERINA YU. DOMRACHEVA, OXANA YU. ILYAKHINA, VLADISLAV YU. DUBROVSKY
- Analyzing the Age Diversity of Patients with Colorectal Cancer** ✱
O.N. ILINSKAYA, M.A. KHARITONOVA, A.N. DOYNIKOVA, B.I. GATAULLIN, P.V. ZELENIKHIN
- Illustration of Experimental version of the Expert System**
RUSTAM BURNASHEV, ARSLAN I. ENIKEEV
- Investigating the Epidemical Infectious Diseases by the Example of HIV** ✱
ARTUR ALMUKHAMEDOV, ILIA VLADIMIROVICH PETROV, FIRUZA SALAVATOVNA PETROVA, LYUDMILA VITALEVNA PETROVA, ALFIR NEBIULLOVICH KHISAMUTDINOV
- Investigating the Consequences Associated with Hepatitis B and C**
ARTUR ALMUKHAMEDOV, ILIA VLADIMIROVICH PETROV, FIRUZA SALAVATOVNA PETROVA, ALFIR NEBIULLOVICH KHISAMUTDINOV
- Treatment Features of Viral Hepatitis B and C in The Russian Federation** ✱
ARTUR ALMUKHAMEDOV, ILIA VLADIMIROVICH PETROV, FIRUZA SALAVATOVNA PETROVA, ALFIR NEBIULLOVICH KHISAMUTDINOV
- The advantages and Disadvantages of Synthesizing of Arylarsonic Acids The advantages and Disadvantages of Synthesizing**
FARID YAMBY SHEV
- The Prediction of Educational Success of Students Humanitarians in Social Networks from the Psychometric View** ✱
FAIL GAFAROV, ZULFIRA ENIKEEVA, GALIM VAKHITOV, KONSTANTIN NIKOLAEV
- Investigating the Epidemiological Features of Tuberculosis in The Republic of Tatarstan and The Russian Federation** ✱
ARTUR ALMUKHAMEDOV, ILIA VLADIMIROVICH PETROV, IRUZA SALAVATOVNA PETROVA, LYUDMILA VITALEVNA PETROVA, ALFIR NEBIULLOVICH KHISAMUTDINOV
- Medical and Social Characteristics of Hepatitis B and C**
ARTUR ALMUKHAMEDOV, ILIA VLADIMIROVICH PETROV, FIRUZA SALAVATOVNA PETROVA, ALFIR NEBIULLOVICH KHISAMUTDINOV
- Phytopathogens' Role in Artificial Greenery in Park Areas of Kazan City**
G.V. DEMINA, G.V. ZAKIROV
- Basic Biological Parameters of White-eye Bream *Abramis sapa***
TATYANA ANDREEVA, VLADIMIR VIATSCHE SLAVOVICH KUZNETSOV, IGOR FEDOROVICH GALANIN, ANNA PETROVNA GALANINA, ZIGANSHIN ILSHAT ILYASOVICH
- Statistical investigation on Effect of Resource- Saving Technologies on Diabetes Mellitus Incidence (A Case Study of The Republic of Tatarstan)** ✱
R. KHAMITOVA, E.R. VALEEVA, N.V. STEPANOVA, V.B. ZIATDINOV, A.R. SABIRZYANOV, D.S. ELAGINA
- Analytical Research on Toxicological Stress on Main Organs**
E VALEEVA, C A ISMAGILOVA, A I ZIYATDINOVA, D.S. ELAGINA, N.S. ARKHIPOVA
- Solving the Problem of Sentiment Analysis Using Neural Network Models** ✱
AYNUR AKHMETGALIEV, FAIL MUBARAKOVICH GAFAROV, FARIDA BIZYANOVNA SITDIKOVA
- Chronic Toxicity Evaluation of Ayurvedic Arsenical Formulation Rasamanikya with Special Reference to Heavy Metal Toxicity.** ✱
NILIMA WADNERWAR, ABHAY PATKAR
- Docking Studies of Lipase (Mflp1) from *Malassezia furfur* (Robin) with Propanamide, N-methyl-2-amino and 1-Propanol, 3-(dimethylamino) Predicting Inhibitory Activity** ✱
A.B. GULWE, D.N MISHRA, K.S. GOMARE
- Docking Studies of Lipase (Mflp1) from *Malassezia furfur* (Robin) with Propanamide, N-methyl-2-amino and 1-Propanol, 3-(dimethylamino) Predicting Inhibitory Activity** ✱
A.B. GULWE, D.N MISHRA, K.S. GOMARE
- Docking Studies of Lipase (Mflp1) from *Malassezia furfur* (Robin) with Propanamide, N-methyl-2-amino and 1-Propanol, 3-(dimethylamino) Predicting Inhibitory Activity** ✱
A.B. GULWE, D.N MISHRA, K.S. GOMARE
- Measurement of Ulnar Variance in Indian population**
DR. ATHIKARI, DR. KALANITHI RAMANUSAN, DR. YESHWANTH SUBASH
- Determinants of Self-Medication Prevention in Women based on the Health Belief Model in Urmia City, Iran** ✱
MORADALI ZAREIPOUR, ZHILA MOHAMMAD REZAEI, FARZANEH JAFARI, SHEYDA GHADERZADH, MONIREH REZAEI MORADALI
- Spectrophotometric Evaluation of Paracetamol in Bulk and Pharmaceutical Preparations** ✱
NASER ATSHAN, HUSAM S. KHALAF

- Determination of Chlorpromazine Hydrochloride in its Pure and Dosage Forms by Diazotization Reaction and Coupling with Diazotized Sulfanilic Acid**
SAHAR FADHEL, ABBAS SHEBEEB AL-KADUMI, WADDAH HILAL ABDULLAH
- Correlation of Calprotectin with Galectin 33, Adropin and CTLA-4 in Iraqi Rheumatoid Arthritis Patients** ✖
SHAKIR T.
- A Review and Analysis of Data Integrity: Pharmaceutical Industry Perspective** ✖
SUMUKHA P, HEMANTH KUMAR S, GANGADHARAPPA H V
- Determination of 1,2-Dibromoethane in Tramadol Hydrochloride Drug Substances by Head Space Gas Chromatography Linked with Mass Spectrometry**
DUGGIRALA VENKATA, KAPAVARAPU MARUTHI VENKATA NARAYANARAO, PULIPAKA SHYAMALA, RALLABHANDI MURALI KRISHNA, KOMALI SIVA PRASAD
- Evaluation of Antilucer Activity of Extract of Calycophyllum Spruceanum (Benth) Bark**
VENKATAIAH GUDISE, DR. I VEENA RANI, P. LAXMI SUMA, R. SHREYA GUPTA, R. BHAVYA SRI
- "Diagnostic Tool for Protein Dependent Disorders through Novel Analytical Approaches"** ✖
ASTHA PATEL, MAHIN PATEL, NILESH PATEL, DHAVALKUMAR PATEL, CHETAN BORKHATARIA
- Edible Seed of Kluwek (Pangium Edule) as Natural Sources of Antioxidant and Antibacterial Compounds** ✖
ACHMAD ZAINUDDIN, IRFAND TRIPUTRA, DINDA FIDIANITA MAHMUDAH, SANDRA AMALIA RIYADI, DIKDIK KURNIA
- A Study on Incidence and Prevalance of Complications in Thyroid Patients at Various Departments of Mamatha Hospital Khammam** ✖
AITHAMRAJU CHANDRA, K. PAVANI, R.SHYAMALA, CHINNA ESWARAIAH.M
- Comparing the Effect of Open and Closed Suction Techniques in Terms of Environmental Contaminant, Cost, and Ventilator Associated Pneumonia Based on the New Definition of National Nosocomial Infection Surveillance System (NNIS)** ✖
SHIRIN AFHAMI, NEGIN ESMAILPOUR, PARVIN REZAEI, SHOKOUH KABIRIAN ABYANEH
- Comparing the Effect of Open and Closed Suction Techniques in Terms of Environmental Contaminant, Cost, and Ventilator Associated Pneumonia Based on the New Definition of National Nosocomial Infection Surveillance System (NNIS)** ✖
SHIRIN AFHAMI, NEGIN ESMAILPOUR, PARVIN REZAEI, SHOKOUH KABIRIAN ABYANEH
- Vesosomes: New Prospects in Multi Compartment Vesicular Drug Delivery System** ✖
KEERTHANA RAJU, GAYATHRI P.S, GAYATHRI KRISHNAKUMAR, SREEJA C NAIR
- Synthesis and evaluation of antibacterial activity of Zinc Oxide nanoparticles**
PARDEEP KUMAR, AJINKYA GIRISH NENE, SHIVANI SOOD, GURPREET KAUR, SANDEEP PUNIA, MANOJ KUMAR, FALAK THAKRAL, HARDEEP SINGH TULI
- Isolation, Identification, and characterization of Microbial that caused Urinary Tract Infection in pregnant women/Iraq**
ASHWAK KZAR
- The Use of Ostrich Eggshell Membrane as a Barrier for Guided Bone Regeneration in Rabbit's Femoral Condyle Defects: Histological Study**
MUSTAFA MUHAMMED, OTHMAN A. OMER, BAKHTIAR MUHIALDIN AHMED
- In-Vivo Shielding Effects of Semecarpus anacardium Extract in Presenile Dementia.**
SAXENA GAURAV, SINGH HANSRAJ, KUMAR NITIN, SELIYA MAMTA, KUMARI NUTAN
- Design and Development of Tacrolimus Niosomal Gel for Treatment of Psoriasis**
VRUNAL V.MORE, PRAJAKTA K.KHULE, MANOJ M.NITALIKAR, RITU M.GILHOTRA
- Immuno-correction of Maladaptation State of the Body of Patients with Pulmonary Tuberculosis in the Astrakhan Region**
I.A KUZNETSOV, RASULOV M.M, KACHANOV I.V, GRIGORIEVA M.A, ANTONOVA M.A
- Cross-Sectional Analysis of Non-Pharmacological Treatment and Glycemic Index Controlled Diet in Management of Prediabetes.**
V.V.N.GOUTHAM, SREYA MANCHALA, MRUDULA VANDANA
- Modification of Diabetic Management Self Effication Scale for the Indonesian Type 2 Diabetes Patient**
YUNI EDHY, E ENGKARTINI, RULLY ANDIKA
- Effect of Blue, Red and Green Colors on Blood Pressure and Heart Rate**
RACHMAT SUSANTO, ELLY NURAHMAH, ATI SURYA MEDIAWATI, SUTANTO PRIYO HASTONO
- The Effect of Nigella Sativa Oil on Number Eosinophils, Neutrophils, Serum Ige Levels to Asthma Mice Resistant of Corticosteroid**
DIAN PALUPI, HARDHONO SUTANTO, UDADI SADHANA, EDI DHARMANA, ENDANG MAHATI, EDIATI SASMITO, SUPRIHATI

- The Impact of 10-Valent and 13-Valent Pneumococcal Conjugate Vaccination on Invasive Pneumococcal Diseases in Children Under Five Year Old: An Article Review**
INGENIDA HADNING, TRI MURTI ANDAYANI, DWI ENDARTI, RINA TRIASIH
- The Challenges and Opportunities of The Anesthesiologist to Improve The Quality of Palliative Care**
ARDI PRAMONO, YUNITA WIDYASTUTI, SUDADI, YATI SOENARTO
- The Diagnostic Test of Ag-TB Rapid Test on Pulmonary Tuberculosis** ✨
INAYATI, ANA MAJDAWATI, SURYANTO
- Anti-inflammatory Activity Effect of Ficus carica and Ziziphus mauritania leaves** ✨
MUHAMMAD KURNIAWAN, MUH INDRRA IRAWAN, ARIFFADLI PRAKOSO, NINING SUGIHARTINI
- Potential Nephroprotective Effect of Valsartan in Renal Ischemia Reperfusion Injury Role of NF-KBP65 Pathway in Rat** ✨
BASIM MOHAMMAD, MAYSAA ALI ABDUL KAHALEQ, MUNTHER ABOSAOODA, ALAA K. MOSA, MOHAMMED A. ABDULHUSSEIN, NAJAH R. HADI
- Nephroprotective Effect of Octreotide and Liraglutide in Renal Ischemia Reperfusion Injury in Mice**
ZAHRAA SALEEM, MAYSAA ALI ABDUL KAHALEQ, MUNTHER ABOSAOODA, NAJAH R. HADI, NASSER GHALY YOUSIF
- Cytotoxicity assay of ethyl acetate extract and isolated rutin and quercetin of Iraqi cultivated Artemisia abrotanum L**
OMAR ALRUBAAE, GHAITH ALI JASIM, ABDULMUTALIB A. NASSER
- Microsatellite instability in uterine cancer**
ALAA ALAM, HAWRAA SAHIB ABID, NAJAH R. HADI
- The Risk Factors of Hepatitis C Virus Infection in Patients with Chronic Kidney Disease on Hemodialysis in Al Amal Dialysis Center-Al Muthanna Governorate-Iraq: A Need for Good Infection Control Methods**
INCE AL, NAWFAL ALI MUBARAK, SAHAR SWADI RAHEEM
- Percutaneous Nephrolithotomy in Children, Results and Complications**
HAYDER ALARIDY
- Detection of biomarkers in serum and synovial fluid of Iraqi patients with knee osteoarthritis**
MOHAMMED ABOOD, MAYSAA ALI ABDUL KHALEQ, ABATHAR A. SADIQ, NAJAH HADI
- Review: Toxoplasmosis: A Review of its Invasion and Immune Response**
KAWTHER M., INAM JOUDAH RAHDHI, HAMEEDAH HADI ABDUL WAHID
- Effect of the Alcoholic extract of the Garlic Plant in the Parasite Entamoeba histolytica in Vivo**
KAWTHER M., INAM JOUDAH RAHDHI, HAMEEDAH HADI ABDUL WAHID
- Study of Bacterial Contaminants and some Chemical Elements of the Euphrates River and Al-Falloja City Drainage**
SHAIMAA DAWD, ATEKA Q. QADDOORI, HANAN F. SALMAN, EBTEHAL M. KALAF
- The Combination of Histopathology and analysis of Accuweather survey of Canine Spirocerosis in Tuwerijarea, Karbala governorate / Iraq**
AMAL ATIYAH, INAM BADER FALIH, ZAIED O IBRAHEEM
- Mycotoxins, Detection and Prevention, A Review**
BALQEES ALI, MUSTAFA SALAH HASAN, ZAID YASEEN IBRAHIM, MAHER SABER OWAIN
- Therapeutic Effects of Olive Oil on Rabbit with Lead Toxicity**
ESSA ALHTHEAL, AMANEE MOHAMMED RADHY, MUSTAFA SALAH HASAN, MOHAMMAD HAMID KAREEM, EMAN NAJI SALEH
- A Novel Portable Smart Prayer Assistant Using RFID**
E. BOOPATHY, C.G. AKALAYA, S. SATHISH, M. MAGESH, D. SANTHOSH
- A Review on Sarcoidin Donkey**
MOHAMMAD KAREEM, RAFID KHALID ALI, INAM B. FALEH, MUSTAFA SALAH HASAN
- Synthesis and Characterization of Some New Cu II, Co II, Ni II, Au III potassium 2-(2,4-dinitrophenyl) Hydrazine-1-Carboodithioate Complexes and Evaluation of their Biological Activity**
GHUFRAN AL-OBAIDY, KAISS R. IBRAHEEM, MOHAMMAD F. MESHER
- Effects of Eucalyptus Alcoholic Extracts on Pathogenic E.coli, Invitro Study**
MOHAMMAD KAREEM, JENAN MAHMOOD KHALAF, MUSTAFA SALAH HASAN, EMAN NAJI SALEH, NOMAN D SALIH
- Synthesis and Biological Activity of New Pyrimidin – Thione Derivatives as Anticancer agent**
RANA ATIYA, ESRAA MOHAMMED, NADA A. SALEH
- Formation, Identification, Microbial Studying of Series Compounds from Chalcone**
ALAA KADHIM, SHIREEN REDHA RASOOL, RAJAA ABDULAMEER GHAFIL
- Synthesis, Spectral Analysis, Chromatographic Studying of Organic Reagents derived from Succinic Acid**
NAGHAM ALJAMALI, SHAYMAA HAMZAH DAYLEE, JALAL HASAN MOHAMMED
- Dynamic Bandwidth Allocation in an SDN Environment Using QoS Queues and**

Open Flow

N. RADHIKA, V. ABHILASH, K.S. NIRANJAN KUMAR, PHANINDRA MEDURI, NIKHIL CHANDRA SAI RAM

A Review in Regenerative Medicine: A Modern Therapy in Horses

MUSTAFA HASAN, OMAR TARIQ HAMMOODI, MOHAMMAD HAMID KAREEM

A Survey on Lung Cancer Early Detection and Classification Using Image Processing Techniques

K. KARTHIKA, G.R. JOTHI LAKSHMI

Avian Candidiasis: A Review

ZAID IBRAHIM, BALQEES HASSAN ALI, RAFID KHALID ALI, AHMED SAMI JARAD, WALEED HAMID FARHAN, MUSTAFA SALAH HASAN

Microwave Synthesis, Characterization of Some Novel Curcumin Compound and its Metal Complexes with Antimicrobial, Antioxidant Studies

ATHRA G.SAGER, AHMED A. SAHEEB, ATHRAA H. MEKKY

Detection Serum Antibodies for TORCH Infections during Aborted Pregnant Women in Fallujah, Iraq

ASIF ABDULRAZAQ, HADEEL MOHAMMED FAYYADH, LAITH M. NAJEEB, KARWAN KHALID KADHAOM

Investigative Accurateness of Ultrasonography and Alvarado Score in Assessing Acute Appendicitis

INVESTIGATIVE OF, DR. KISHOR V. BANDAGAR, DR. CHANDRASHEKHAR Z. PARDESHI

Assessment of Risk Factors Involved with Video Display Terminal Syndrome

DR. NIRAV, DR. GIRISH A. GADRE

A Randomized Control Trial of Adenotonsillectomy with and without Insertion of Grommet in Treatment of Secretory Otitis Media

DR. SINGH, DR. VIKAS KULKARNI

Evaluating Sealing Ability of Three Different Furcation Perforation Repair Materials

DR. MATIGATTI

Efficiency of erythropoiesis-stimulating medicines of renal anemia on the background of hemodialysis

BATIR DAMINOV, AQIDA DJABBAROVA, AZIZA IKRAMOVA, BARNO UBAYDULLAEVA

Place of endothelial dysfunction in the formation of obstructive pulmonary disease in arterial hypertension

NAFISA SHAMUKHAMEDOVA, GULCHEKHRA KADIROVA, BOTIR DAMINOV

Using modern radiologic methods in the early diagnosis of acute hematogenic osteomyelitis of the bones of the hip joint in children

JAMSHID SHAMSIEV, NUSRAT BOYJIGITOV, NEMAT BOYMURODOV, ERKIN DANIYAROV, SUKHROB ZAINIEV, ZAFAR MAKHMUDOV, AZAMAT SHAMSIEV

Development of technology for evaluation and formation of educational and behavioral education and personal characteristics of students of the higher medical educational institution

LAZIZ TUYCHIEV, FERUZA TUKHTAKHODJAEVA, ERKIN ERMETOV, MURATALI BAZARBAEV, AKHMAD MARASULOV

Morphological status of the liver and some aspects of free radical processes in experimental brain ischemia

ABZALOVA SHAKHNOZA, IKRAMOVA SURAYYO

Morphological features of ischemic and hemorrhagic brain strokes

KAMALOVA M.I., ISLAMOVSHE

The priority of vertebroplasty for treatment of symptomatic vertebral hemangiomas

AZIZ QOBILOV,

Interactive component of non-traditional lectures in post-diplomacy education at dentistry departments

ULUGBEK MUSAEV,

Is the megareuter the problem of yesterday, today or tomorrow?

YU.M. AKHMEDOV, G.S. KARIMOVA, I.YU., AKHMEDOV

Surgical correction of genital prolapses in women of reproductive age

NIGINA NASIMOVA,

Preoperative mistakes in the surgical treatment of upper retro micrognathia

JASUR RIZAEV, AZIZ KUBAEV

Anemia during pregnancy

FOTIMA ASKAROVA, KUDRAT ASKAROV, NILUFAR ASKAROVA

Systemic cytokine production in patients with bronchial asthma depending on the clinical-pathogenetic phenotype of the disease

KH.I. TURDIBEKOV, R. KH. KHOLLIEV, SH.KH. ZIYADULLAEV

Synthesis of polymeric derivatives of lactic acid by chemical transformations of polyacrylamide

SEVARA KHAZRATKULOVA, MAKHKAMOVA LATOFAT, NIGORA ZULYAROVA

HLA - COPD and bronchial asthma markers: commonalities and differences

N.R. ARALOV, G.A. DUSHANOVA, SH.H. ZIYADULLAYEV

Optimization of diagnosis and treatment tactics of early biliary complications after

cholecystectomy*Z.B. KURBANIAZOV, I.A. ARZIEV***Significance of $\beta 2$ -adrenoreceptor gene polymorphism molecular genetic mechanisms of formation of bronchial asthma***HUSAN TURDIBEKOV, RAMZIDDIN HOLLIYEV, SHUKHRAT ZIYADULLAEV***Surgical treatment tactics for patients with combined pelvic and femoral injuries***HASAN TILYAKOV***The tactic of surgical rehabilitation of the orbicular ligament with dislocations of the radius bone head in children***SHERZOD ERANOV***Features of perioperative management of children with diffuse appendicular peritonitis***SHUKHRAT YUSUPOV, KARSHIBOY BABAYAROV, ABDIKODIR SHAKHRIEV***Neuroimmunological aspects of pathogenesis in children's cerebral palsy***AZIZA DJURABEKOVA, GULJAHON UTAGANOVA, SAODAT IGAMOVA, AKMAL GAYBIYEV***Modern issues of diagnosis and treatment megaureter in children***SHUKHRAT YUSUPOV, SHOKHRUKH YUSUPOV, AZAMAT SHAMSIEV***Influence of various treatment regimens on functional – oxidation dysfunction and hipoxycelle cutanea in patients with chronic obstructive pulmonary diseases and parodontitis***JASUR RIZAEV, DILOROM RAKHIMOVA, SARVAR JUMAEV***Complication of encephalitis in children, an innovative approach for the treatment***S.T. NIYOZOVH, S. ?. IGAMOVA, A.T. DJURABEKOVA***Analysis of aspirated foreign bodies of the respiratory ways in children***JAMSHID SHAMSIEV, OTABEK IBRAGIMOV, KARSHIBOY BOBOYAROV, SUKHROB ZAINIEV, ABDURAUUF ISAKOV, JASUR RUZIEV, AZAMAT SHAMSIEV***Profile of pro-inflammatory interleukins in symptomatic epilepsy of various etiologies***YAQUTHON MADJIDOVA, MAVJUDA MUKHITDINOVA, NODIRA KHAMIDOVA, VASILA ABDULLAEVA, GULCHEKHRA SADIKOVA, ALIE KHALILOVA***Innovative methods for diagnostic of pain syndrome in patients with trigeminal neuralgia***SITORA INOYATOVA, SHAKHLO MUKHAMMADSOLIKH, AZIZA DZHURABEKOVA, NARGIZA ABDULLAEVA, KOMILAKHON NASIRKHODJAEVA, YAKUTKHON MADZHIDOVA***The role of apoptosis in the development of acute pancreatitis***RIKHSI SABIROVAH, ESTELLA KALENDAREVA, NURKHON MUKHAMEDOVA, ALISHER GANIEV***Age, Duration of Marriage, Years of Treatment, Anger Experience and Expression among Infertile Women in the Treatment***DEVIGA SUBRAMANI, DR. MAYA RATHNASABAPATHY***Probabilistic Neural Network for Automatic Detection of Plant Diseases Using DT-CWT and K-Means Feature Extraction***V. SRIYA, DR.T. MANORANJITHAM, R. ISHWARYA***Fixed Point Theorems in Normed Linear Space of Triangular Fuzzy Numbers***G. VEERAMALAI, K. CHITIRAKALA***Application of Fuzzy Graph in Social Networking***VINITHA VARUVEL, A. KANCHANA***Prevention of Non-Proliferative Diabetic Retinopathy (NPDR) by Detecting Exudates, Sub-conjunctival Haemorrhages and Micro-aneurysms***K. MANJU, KOLLA SRUTHI, P. POOJA KARAN, M. POOJA, M. AMUTHA***Effect of Rainfall on Groundwater Quality in the Delhi-NCR Region***PIYUSH GHIYA, DR.S. ANBU KUMAR, PRATIK JOSHI, SAMIKSHA SHRIVASTAVA***Promotion Response Modelling in Healthcare Analytics***ASHUTOSH SHANKHDHAR, SUYASH DIXIT, ARUSHI MANGALA, AYUSH GUPTA, VINAY AGRAWAL***An Effective Service Recommendation Mechanism Using Collaborative Filtering and Machine Learning***ASHUTOSH SHANKHDHAR, ROHAN BHARDWAJ, SATYAM GUPTA, RAVI VYAS, PIYUSH KUMAR***Detection of Pothole and Warning System***M.N. DIVYA, B.K. NANDEESH, NAGARAJ S NAREGAL, K.B. GURUPRASAD, ANUP G KOPARDE***FPGA based Analysis of Electro Cardiography Signals Utilizing Fir Channels***K. LIKHITHA, S. JAYANTHI***Prediction for Type of Mobile Application Based on User's Behavior***S. SRIVIDHYA, SORNALAKSHMI, S. SINDHU, S. NITHIYA***Online Subjective Answer Checker***R. AISHWARYA, M. TANUSHA, M. HARSHITHA***Assessment & Forecast of Air Quality data based on Neural Network***R. AISHWARYA, K. SOWJENYA, SRAVANAPU DEEPIKA***Blind-Spot Monitoring Using Deep Learning**

T. PONNARASAN, M.G. SUMITHRA, I. RUBAN, T.R. RANJITH KUMAR

Voting System based on Finger Print and Face Recognition

T. SHANTHI, S. SRIPRIYA, K. SRINANDINI, S. SELVASRIDEVI, R. SARANYA

Nautical Alert System (NAS) Using Reverse Motor Mechanism in IOT

RAVI SRIDHARAN, G.V.S.N. SRI HARSHA, D. HEMA SAI KUMAR, D.V. SAI PRAKASH, J. THANGAKUMAR

Accurate Object Detection with YOLO

J. AVANEESH, J. THANGAKUMAR, T. SUDALAIMUTHU, P. RANJANA, N. SATYA PRAKASH, N.V.V. SAI TEJA

Cognitive Socio Robot with Temperature Sensor for Environment Protection

P. SAITHARUN, DR. AMUDHA

Prevalence of Hepatitis C and B Virus Infection among Patients Undergoing Blood Transfusion and Dental Treatment in Al-Najaf Governorate

ELHAM KADHUM

Formation, Characterization, Basic Studies and Biological Effect of Mixed Ligand Complexes of Cu-ion and Ni-ion Derived from Azo Compounds, Schiff bases and (1,10, phen)

LAYLA. MOHAMMED, MUNA ABASS HADI, MITHAQ SAEED MOHAMMED

Problems Encountered by Female Faculty in Achieving Work-life Balance

P.S. PRATHIBHA, M. PRABHU, DR.B. NEERAJA

Effect of Feeding Diet Containing Probiotics on Growth Rate and Hematological Changes in the Blood of Turkeys

AIRAT KHABIROV, INNA SAMORODOVA, ALEXANDER KUZNETSOV, IRINA LYKASOVA, NATALYA FEDOSEEVA, MARINA DERKHO, OLGA GORELIK, YAROSLAV REBEZOV

Chemical Composition, Antioxidant and Anti-Inflammatory Activity of Ficus Racemose Fruit

MALA RAJENDRAN, KEERTHANA RAVI CHANDRAN, KARTHIKEYAN RAMESH, RUBY CELSIA ARUL SELVARAJ

Detection of lmrS, mepA, norC genes among Staphylococcus Aureus Isolates

MARWA JEAD, RANA KADHIM MOHAMMED

Investigating Nursing students' needs and data sources in the emergency wards

MASOOMEH MAHDAVIFAR, NEDA ASADI, FATEMEH SHADI, NILOUFAR CHOUBIN, MOHSEN AZAD, FOROOGH ZIARATI

Motor and F wave hyperexcitability in Neuromyotonia: A rare observation

CHATURBUJ RATHORE, SANJAY PRAKASH, DEEPAK BANDLISH, KAUSHIK RANA

Prevalence and Severity of Vitamin D Deficiency in Hypothyroidism- A Case Control Study

DR PANDYA, DR DHRUV KOTECHA, DR ROHIT CHORDIYA

Development of first-order Derivative Spectrophotometry for Simultaneous

Determination of Domperidone and Ilaprazole

DILLIP DASH, ASHIM KUMAR SEN, AVINASH KUMAR SETH, S. SAISIVAM, SHIVKANT PATEL, PIYUSH KUMAR SADHU

Diclofenac Induced Acute Kidney Injury: A Case Report

VIKAS CHANDRAKAR, MAHEK MISTRY, HEMRAJ SINGH RAJPUT, SUNIL BHAURAO BAILE, DHANYA B SEN

Two Spectrophotometric Methods for the Simultaneous Analysis of Nadifloxacin and Mometasone Furoate in Cream

AARTI ZANWAR, DHANYA B. SEN, AREZZ ATIQUE VORA, RAJESH A. MAHESHWARI, ASHIM K. SEN, A.K. SETH

Association between treatment needs of periodontal status and systemic diseases among 35-80-year-old adults attending a private dental college in Chennai - A Retrospective study

LALITHA RANI, MEIGNANA ARUMUGHAM INDIRAN, JAYASHRI PRABAKAR

Predictivity of mandibular third molar position as risk indicator for pericoronitis, Pericoronal abscess formation.

YANDETI SRINIVASULU, ABDUL WAHAB .P.U, SENTHIL MURUGAN.P

Occurrence of salivary gland malignancies among an outpatient population visiting a dental college

CIBIKKARTHIK T, GHEENA S, JESSY P

Awareness on the recent advances in the treatment of lymphoma- A survey

SRIVATCHAVA SELVARAJ, A. JOTHI PRIYA, V. VISHNU PRIYA

Denture base adhesive usage- A Survey

B VIVEK, DHANRAJ GANAPATHY, SAMUEL RAJ SRINIVASAN

Surgeons preference for techniques and materials for suturing and its influence on healing after flap surgery

PRASHAANTHI.N, SHEEJA S. VARGHESE, SHANTHA SUNDARI.K. K

Choice of root canal irrigants used amongst post graduates for treatment of immature non vital permanent teeth in pediatric patients visiting a dental college.

SHEBI S, MEBIN GEORGE MATHEW, SURESH. V

Awareness and practice on various restorative and non restorative treatment options for incipient carious lesions among undergraduate students

RAJA KUMAR, SINDHU RAMESH, V.VISHNU PRIYA

Maxillofacial Trauma And Pathology Among Paediatric Patients - A Retrospective

Institutional Experience*SHIVANGI GAUR, MADHULAXMI M, HEMAVATHY O R***Suicide In Adolescents: Mental Health Problems And Social Support***FLORENSA , BUDI ANNA KELIAT, IRMANSYAH, BESRAL***The Effect of Aerobic Exercises on Cognition, Physical Fitness, and****Apolipoprotein E Plasma among Women with Mild Cognitive Impairment**
*, LISNA ANISA FITRIANA, SUCI TUTY PUTRI, KUSNANDAR ANGGADIREDA, RIA MARIA THERESA, I KETUT ADNYANA***Healthy Pregnancy Preparation through Pre-marital Guidance Model on Prospective Brides***SITI ROFIAH, SRI WIDATININGSIH***Correlation Between The Expression Of Egfr And Pd-L1 With Prognosis Of****Glioblastoma Multiforme Without Idh Mutation Treated With Nimotuzumab**
*PATRICIA DIANA, LISYANI, SUROMO, UDADI SADHANA, HARDHONO SUSANTO, INDRA WIJAYA, CAHYONO KAE LAN***An evaluation of social media on academic performance of college students in online classes and examinations during lock down period.***SRIDEVI.G , NANDITA.R, SRIVIDYA.S***Assessment Of Knowledge, Attitude, Practice Based Survey On Awareness Of Emla Among Dental Professionals***SOYA XAVIER, DR.ABDUL WAHAB, DR.M.SIVAKUMAR***Analysis Of Various Impression Techniques Used In Fixed Partial Denture Among Dental Students - A Retrospective Analysis***FAHMIDA ABD, DHANRAJ GANAPATHY, SUBHASHREE R***reconstruction of deficient maxillary alveolus with various guided bone regeneration techniques - a case series***DR.KABILAMURTHI R.S, DR.SUBHASHREE R, DR.THIYANESWARAN N, DR.RAJENDRA PRABHU ABHINAV***Management Of Complicated Crown-Root Fracture By Surgical 180-Degree Rotation: Case Report With One Year Follow-Up***DIVYA SUBRAMANYAM, DEEPA GURUNATHAN, MEBIN GEORGE MATHEW***iPad based training programme to teach tooth brushing among children with autistic spectrum disorder***PRERNA JAIN, DR.MEBIN MATHEWS***Knowledge, Attitude And Practice Among Private Dental Practitioners About Usage Of Periodontal Diagnostic Instruments And Pre Procedural Rinsing - Kap Study***SUBASREE SOUNDARAJAN, JAIGANESH RAMAMURTHY***Awareness On Management Strategies For Halitosis Among Dental Students***TASLEEM S, DHANRAJ GANAPATHY, SUBHASHREE R, RAKSHAGAN V***Knowledge, Attitude & Practice Survey On Digital Dentistry Among General Dental Practitioners***KALYANI BEHERA.A, IFFAT NASIM***Virtual screening to identify virulence factors in red complex pathogens targeted by naringin.***LASYA GENJI, SMILINE GIRIJA A.S, VIJAYASHREE PRIYADHARSINI.J***Anti-inflammatory activity of Acacia catechu bark extract against raw 264.7 macrophage cell line***LAKSHMI.T , ANITHA ROY, KAVIYA N.E***Awareness Among Dental Students On Strategies Over Early Childhood Caries.***T.USHANTHIKA , DHANRAJ GANAPATHY***Evaluating The Efficacy Of Abgel Soaked In Manuka Honey Following Extraction Of Infected Mandibular Molars***SNEHA KRISHNAN, SENTHILNATHAN PERIASAMY, MURUGAIYAN ARUN***Knowledge And Awareness About Snoring Habits Among Healthy Individuals***AARTHI KANNAN, R.GAYATRI DEVI, A.JOTHI PRIYA***Knowledge, Attitude, And Practice Regarding Standardized Treatment Protocol For Pulp Therapy In Deciduous Dentition Among General Dental Practitioners Of Chennai, India- A Questionnaire Survey.***ADITYA JAIN, GEO MANI***Knowledge, Attitude And Practices About Food Habits Among Lactating Women***ANKITA LABH, R.GAYATRI DEVI***Awareness Of Management Of Carcinoma Of Maxillary Antrum Among Dental Students***KEERTHANA BASKAR, DHANRAJ GANAPATHY***Study Of Incidence And The Distance Between MB1 And MB2 Canals In Maxillary Molars Using CBCT Analysis***MONICA ANTONY, ANJANEYULU K***Estimation Of Blood Pressure Among Healthy And Constipation Individuals***NGOBINAH PRETTY, R.GAYATRI DEVI, A.JOTHI PRIYA***Awareness Of Management Of Malignant Melanoma Among Dental Students.***NANDITA R, DHANRAJ GANAPATHY.L, KEERTHI SASANKA***Morphometric Analysis Of Kawase's Triangle And Its Clinical Application Using Human Dry Skull Bones**

RITHANYA.P , KARTHIK GANESH MOHANRAJ

Morphometric Analysis Of Tympanic Plate , External Acoustic Meatus And Mandibular Fossa In South Indian Dry Skull

DHIVYA SRI.E, DR.DINESH PREMAVATHY

Diet And Other Factors Affecting Non Habit Oral Cancer Patients

CHITHRALEKHA B, GHEENA S

Smoking Prevalence and Behaviour among College Students in Chennai - A Survey based study.

UMAYAL.S , PRADEEP KUMAR.R

Management Of Tissue Emphysema In Endodontics - Decision Tree Analysis

KRISHNAKANTH JAJU, IFFAT NASIM

Incidence of Meningo Orbital Foramen in Dry South Indian Skulls and its clinical significance

P.HARINI , M.S THENMOZHI, DR.DINESH PREMAVATHY

Assessment of Dental Anxiety During Endodontic Treatment for Primary Teeth in Children

ANUPAMA DEEPAK, GANESH JEEVANANDAN

Knowledge, Awareness, and Practice of 3D printing in the field of conservative dentistry and Endodontics

NAUMA HAFEEZ, MANISH RANJAN

Oral Hygiene Maintenance In Children - A Survey On Parental Awareness

PALAK SHAH, GANESH JEEVANADAN

Knowledge, Attitude And Practice Regarding The Restoration Of A Badly Broken Root Canal Treated Tooth

PAVITHRA DAVE, MANISH RANJAN

Knowledge, Awareness And Treatment Options For Gingival Pigmentation- A Survey

HEMASHREE J, KAARTHIKEYAN G

Assessment of anxiety among dental students performing extraction and patients undergoing third molar extraction

SHRUTHI DEVI.R, M.JEEVITHA, PREETHA.S, KARTHIK GANESH MOHANRAJ

Modified Obwegeser's Vestibuloplasty : A Case Report

R RAJASHRI, SANTHOSH KUMAR M.P, PROF.DR.M.R.MUTHUSEKHAR

Knowledge and Awareness on the Application of 3D printing in Endodontics

RESHMA THIRUNAVAKARASU, MANISH RANJAN

Knowledge, Attitude And Prevalence Of Tobacco Among Government And Private High School Children In Trichy District: A Cross Sectional Study

S.SUSHANTHI , PRADEEP KUMAR.R

Knowledge and attitude regarding cord blood banking and donation among pregnant women in Chennai - A Questionnaire Survey

S.VYSHIALI , D.SRI SAKTHI, I.MEIGNANA ARUMUGHAM, R.PRADEEP KUMAR

Knowledge And Awareness About Distraction Osteogenesis Among Dental Students

THANISH AHAMED.S, DHANRAJ GANAPATHY, SUBHASHREE.R

Prevalence Of Severe Early Childhood Caries And Associated Risk Factors Among 12-36 Month Old Children

VARUSHA CHRISTOPHER, GANESH JEEVANANDAN

Awareness On Oral Hemangioma Among Dental Students- A Kap Survey

VARUSHA CHRISTOPHER, DHANRAJ GANAPATHY, SUBHASHREE R, RAKSHAGAN V

Viral Hepatitis in Dhi-Qar Province: Demographics and Hematological Characteristics of Patients

ABDULADHEEM JALIL, SAJA HUSSAIN DILFY, ALEKSANDER KAREVSKIY, NAWRAS NAJAH MUBARK

Association of EBV with Hodgkin and Non-Hodgkin Lymphoma for Females and Males Patients: Histological, Molecular and Survey Study in Karbala, Iraq

, , ABEER CHEAID YOUSIF AL-FATLAWI

Molecular Typing and Antimicrobial Susceptibility Patterns of Escherichia coli Isolated from patients with urinary tract infection.

AHMED REMTHAN HUSSAIN, MANAL BADI SALEH

Renal Impairment in Diabetes Mellitus Type2 In Iraq

ALAA ABDULJABBAR ABDULRAZZAQ

Preterm premature rupture of membranes management with erythromycin versus azithromycin

ALAA IBRAHIM ALI

An Evaluation of Receiver Operating Curve (ROC) For ABCG5/G8 Transporters Serum as Diagnostic Test in Patient with Hepatobiliary Diseases

ANTESAR RHEEM OBEAD, LECTURE.RANA.S.AL-SHEMARY, HAIDER RASOL ABBASS ALWAHAMI

The Role of Interleukin-33 in Chronic Viral Hepatitis B Patients

AQBAL SUHAIL NAJM ALJIZANI, ALIA ESSAM MAHMOOD ALUBADI, SAFAA ABDUL-KARIM ABDUL-RAZAK, ABBAS MAHMOOD AHMED

The relationship between the prolactin level and kidney function in women with

- hyperprolactinemia**
BUSHRA ISMAIL IBRAHIM
- Determination of Virulence Factor Activity of Pathogenic Candida Collected from Variety Specimen Source.**
FAKHRI S.ALAJEELI
- The Relationships Between Acetylcholinesterase Activity and Some Ions (Calcium, Sodium) In Patients Diabetic Mellitus with Neuropathy in Babylon Province**
FIRDEWS Y.HAMZA, ODA M. YASSER
- Cardiovascular Disorders and Obstructive Sleep Apnea**
AFRITA AMALIA LAITUPA, R P SOEHARSOHADI TJONDRONEGORO
- Diagnostic Imaging of Silicosis**
AFRITA AMALIA LAITUPA, WINARIANI KOESOEMOPRODJO, MOHAMMAD YAMIN SUNARYO SUWANDI
- Respiratory Failure in Massive Tuberculosis Hemoptysis**
AFRITA AMALIA LAITUPA, WILY PANDU ARIAWAN, DANIEL MARANATHA, PRASENOHADIA, MENALDI RASMIN
- Is Facilitated Tucking by Parents More Effective than Dextrose Water (10%) in Reducing Full-Term Neonatal Pain during the Heel-Lancing Procedure: A Randomized Controlled Trial**
DR.MANAL KASSAB, DR. KHAWLAH NUSEAIR, NOOR AL-QAOU, DR. SHEREEN HAMADNEH, ANIT N ROY
- The Effect of Home Pharmacy Care Education on the Quality of Life of Diabetic Patients in Community Health Care**
PINASTI UTAMI, MT GHOZALI, BANGUNAWATI RAHAJENG, SUNDARI
- Brain Computer Interface (BCI): Mechanism and Challenges - A Survey**
DIPANNITA DEBASISH MONDAL, DR.MUKIL ALAGIRISAMY
- The Efficacy Test of Nano Chitosan and Phylex in Resistance Early Blight Disease in Tomato Caused by Alternaria Solani Fungus**
QASIM ATIYAH ABD AL-TAMIMI, HALIMA ZUGHER HUSSEIN, AMNA MUHAMMAD ALI
- MILA In Teaching Oral Pathology**
DR. NALLASWAMY, DR.M. SUBHA, DR. PRATIBHARAMANI, DR. HERALDSHERLIN, DR. GHEENA.S, DR. ABILASHA.R, DR. GIFRINA.J, DR.DON. K. R, DR. ARCHANASANTHANAM, DR. MONIKA.K, DR. HANNAH.R
- In vivo Analysis of The Alteration of Salivary Ph By Chewing Fennel Seeds in Caries Patient**
KOWSHIHAN P, MURALIDHARAN N.P
- Awareness on Association of Irregular Eating Habits and Skin Problems Among Women - A Survey**
MEENAPRIYA M, R.GAYATHRI, V.VISHNU PRIYA
- Comparative Assessment of Intraoperative Pain During Crown Lengthening Procedures Using Soft Tissue and Hard Tissue Laser**
CHRISTEENA ABRAHAM, SANKARI MALAIPPAN
- Effect of Dental Chair Light on Bond Strength of Composite Used for Bonding of Brackets - An In Vivo Study**
NILESH SURESH, SHANTHA SUNDARI KK
- Anatomical Study of Important Anatomical Landmarks in The Skull in Relation to External Occipital Protuberance**
ALDRIN A, DINESH PREMAVATHY
- Morphometric Study of Distal End of Humerus Bone in South Indian Population**
P.ANUSHYA , SANGEETHA.S
- Morphometric Study Of Lower End Of Dry Femur In Indian Population And Its Clinical Implications**
S.SELVAPRIYA , S.SANGEETHA
- Evaluation of Sexual Dimorphism Using Morphometric Analysis of Clavicle**
KEERTHANA B, S.SANGEETHA
- Awareness About Dentin Hypersensitivity and Its Various Treatment Methods Among Dental Students**
ANJANA GOPINATH, L.LEELAVATHI
- Awareness on Impact of Electronic Media on Children's Growth and Development Among Parents**
ASHNA YUVARAJ, DR.S.PREETHA, DR.LAVANYA PRATHAP, DR.M.JEEVITHA
- Knowledge and Awareness on Impact of Insufficient Sleep-A Productivity Killer, Among Dental Students**
JANANI K, DR.S.PREETHA, DR.LAVANYA PRATHAP, DR.M.JEEVITHA
- Piezo Surgery in Effective Management of Resorbed Alveolar Ridges - A Case Series**
DR.KABILAMURTHI R.S, DR.SUBHASHREE R, DR.THIANESWARAN N, DR.RAJENDRA PRABHU ABHINAV
- Comparative Evaluation of Total and Free Sialic Acid in Saliva of Smokers and Tobacco Chewers to Determine Sialic Acid as A Salivary Biomarker for Oral Cancer**
JOHN FRANCIS, DR.J.SELVARAJ, DR.V.VISHNU PRIYA, DR.R.GAYATHRI
- Morphology and Morphometry of Carotid Canal in South Indian Skulls**

HARINI M, LAVANYA PRATHAP, PREETHA S

Antioxidant and Antihypercholesterolemic Potential of Methanolic Root Extract of Salacia Oblonga- An In Vitro Study

V.A.MURALIDHARAN , R.GAYATHRI, V.VISHNU PRIYA

Association of Height and Foot Length Measurement in Normal Population - An Anthropometric Study

ABINAYA.S , LAVANYA PRATHAP, PREETHA S

Effectiveness of Train the Trainers Program in Improvement of Oral Health of Children in Tamilnadu – A Randomized Trial

LALITHA CHELLAPPA, SS RAJ, MEIGNANA ARUMUGHAM INDIRAN, PRADEEP KUMAR RATHINAVELU

Dental Health Status Among Elderly Orthopaedic Patients

A.ASHWATHA PRATHA, R.GAYATHRI, V.VISHNU PRIYA

Comparative and Morphometric Study of Lateral and Medial Malleoli

B.MADHUMITHA , S.SANGEETHA

Typodont Vs Natural Teeth for The Practice of Pre-Clinical Conservative Dentistry - A Survey

FARHAT SADIQUE, ANJANEYULU K

Morphometric Analysis of Foot Among Young Adult Population

K.JANANI , S.SANGEETHA

Fsh Status in Individuals with Polycystic Ovarian Syndrome

G DEVI, ROY ANITHA, SAVITHA G, T LAKSHMI

Knowledge Attitude and Perception of Patients Towards Orthodontic Treatment- A Questionnaire Survey

SRIJAN SUNAR, NIVETHIGAA,B

Awareness of Oral Hygiene Practice in Prevention of Periodontal Diseases Among South Indian Population

PRANEESHA.R , LAVANYA PRATHAP, PREETHA S

Existence of Foramen of Huschke In Human Dry Skulls

RAJA KUMAR, LAVANYA PRATHAP, PREETHA S

In Vitro Antidiabetic Potential of Aqueous Cladode Extract of Opuntia Dillenii

SARVASRI T, R.GAYATHRI, V.VISHNU PRIYA

Awareness of Dental Fluorosis Among College Students in Chennai - A Survey Based Study.

SHARON KEZIAH.V, KANIMOZHI.G, R.PRADEEP KUMAR

Comparative Evaluation of The Effect of Amoxicillin and Doxycycline as An Adjunct to Non-Surgical Periodontal Therapy in Reduction of Inflammation Among Generalised Chronic Periodontitis Patients

SIDDHARTH NARAYAN, SANKARI MALAIAPPAN

Assessment of Bolton's Ratio Including the Second Molar Using 3shape Software

ANJALI THOMAS, SRIRENGALAKSHMI M

Evaluation of Antidiabetic Potential of Crude Methanolic Leaf Extract of Gymnema Sylvestre - An In Vitro Study

JAYADHARANI , R.GAYATHRI, V.VISHNU PRIYA

Awareness and Attitude Towards Missing Tooth Replacement Among Partially Edentulous Outpatients - A Hospital Based Study

LAKSHYA RANI.S, L.LEELAVATHI

Human Red Blood Cell (Hrbc) Membrane Stabilization Potential of Crude Methanolic Leaf Extract of Gymnema Sylvestre - An In Vitro Study

LEKHA.D , R.GAYATHRI, V.VISHNU PRIYA

Protection of human rights in the context of combating of counterfeiting medicines: theoretical and legal issues of international cooperations

VASYL V.ILKOV, YURII V. FILEI, PAVEL G. DAVIDOV, DMYTRO YERMOLENKO, HANNA KOVERZNIIEVA

Study of hormonal status in infertile women with hyperandrogenemia depending on AMH levels

GURBANOVA J.F., GAZIYEVA N.C., ABDULLAYEVA A.A., MAMMADOVA R.M., KERIMOVA T.T., BAGIROVA G.R.

Social Control of the Tendency of Migration from Chinese Post-Substantial Rural Community

WANG LILI, LIANG JIAN, LI ZHONGYUAN

Right to health protection in the former-soviet constitutions: constitutional and legal analysis

MAKSAKOVA RUSLANA, YURII VIKTOROVYCH KYRYCHENKO, MARYNA LEONYDOVNA SMOLIAROVA

A new approach to the treatment and prevention of atopic dermatitis. Possible way of crisis resolution.

SERGEY F.GLADKOV, NINA K. PEREVOSHCHIKOVA, YULIA S. PICHUGINA, MARINA A. SURKOVA

Information security and social work to counteract cultural and information wars in neo-functionalistic reflection

PRODANIUK ROMAN, ROMANENKO YURIY VIKTOROVICH, POGREBNAYA VIKTORIYA LEONIDIVNA, HORDIENKO NATALIIA, BEKDAMYROVA LIDIYA ZAUROVNA

Functional State of Mastication Muscles in Patients During Treatment of Lower Jaw Defects with Implants

MANSUR SH. MIRZOYEV, KHURSHHEJAN ?. GAPHAROV, DODARJON I. KHUSHVAKHTOV, MUKHAMEJAN N. SHAKIROV, ROZIYA N. JONIBEKOVA, RUSTAM I. YULCHIEV

Treatment of Adolescents with Congenital Lateral Incisor Edentia, Use of Aligners and Bracket Systems.

PILIPENKO , BAZILEVICH, MAKSYUKOV, PILIPENKO

Nasonex in the Treatment of Complications of Allergic Rhinitis After Rhinoplasty Relevance.

BAMMAT I. DZHANTEMIROV, AMINAT A. OMAROVA, KARINA YA. SHUGGEVA, FARIZA D. AKHMEDOVA, ALBINA S. ABASOVA

Liability For Aggression Against Human Health

, , NOSIRJON KOMILOVICH HOJIYEV

Reasons for Illegal Actions of Medical Personnel and The Conditions That Allowed It

SHUKHRATJON JUMAEVICH KHAYDAROV

Enhanced Antifungal Activity of Voriconazole-Loaded Solid Lipid Nanoparticles and Nanostructured Lipid Carriers Against Aspergillus Flavus Isolates from Goat

ALWAN A. H. AL QUSHAWI, KHAIRI JAMEEL AL-RUABY

Educational Inclusion in Times Of COVID-19: Talking with Female Inclusive Teachers in Lima-Peru

EDITH GISSELA RIVERA ARELLANO, JUAN MÉNDEZ VERGARAY, YOLANDA JOSEFINA HUAYTA FRANCO, LUZMILA LOURDES GARRO-ABURTO, ENAIDY REYNOSA NAVARRO

A Study of The Macro and Micro Vascular Complications in Diabetics Attended the Diabetes Center of Al-Sader Medical City

HASANAT ALJABERY, LAITH ALI UNISE, AHMED HASSAN KUDHAIR, AHMED MOUSA ISSA

Authentic Leadership as An Intermediary Variable the Relationship Between Learning Organization and Lecturer Performance

TEDY ARDIANSYAH, ENDRY BOERISWATI, USEP SUHUD, NURJANAH, TJIPTO DJUHARTONO, DEDDY DARIANSYAH, AGUS SUPANDI, AHMAD YANI

The Influence of Personality Type on Depression and Anxiety in Anaesthesiology Residents

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The Effect of Aerobic Exercise Accompanying a Diet Program on Some Anthropometric Measurements for Women (35-45) Years Old

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