Heterocyclic Letters
Vol. 9| No.2|165-176|Feb-April |2019
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

TO STUDY THE ANALGESIC, ANTIOXIDANT , ANTIFUNGAL & ANTI-INFLAMMATORY ACTIVITY OF SYNTHESIZED DIHYDROPYRIMIDIN-2(1H)-ONES

Roshani R. Dharamkar*[1], Rahimullah Shaikh[1], Mohammad Shoeb[1]

Department of Chemistry,
Govt. Vidarbha Institute of Science and Humanities, Amravati, Maharashtra,444604- India
E-mail: roshanidharamkar@gmail.com

Abstract: To evaluate and compare the antioxidant potential and anti-inflammatory activity Reducing power assay method for determination of antioxidant activity/potential of samples.The medium used for the study of antimicrobial activity of the newly synthesized compounds was Hi-Media Laboratories Pvt. Limited, India. . The antibacterial and the antifungal agents having the property of inhibiting bacterial or fungal multiplication are called as bacteriostatic or fungistatic.
The substance or compound to be evaluated must be brought in an intimate contact with the test organisms against which activity is to be estimated.Favorable conditions like nutritional media, temperature, incubation time etc. must be provided to offer a maximum opportunity for optimum growth of the organisms in absence of antimicrobial agents.The synthesized compounds are subjected to invitro inhibition of protein denaturation in various concentration i.e. 100, 200, 400, 800, 1000 ug/ml.

Keywords: Analgesic, Antioxidant, Antifungal, Anti-inflammatory activity, DPPH, Total phenolic content.

Introduction:

The reducing property is generally associated with the presence of reductants. The antioxidant actin of reductants is based on the breaking of free radical chain by donation of a hydrogen atom. Reductants also react with certain precursos of peroxide, thus preventing peroxide formation. The presence of antioxidant molecule in sample act as reductants by donating the electrons and reacting with free radicals to converts them to more stable products and terminate radical chain reaction.
According to WHO, More than 80% of the population within developing countries uses herbal and other traditional medicines to treat their common ailments[1]Nature has bestowed Oman with an enormous wealth of medicinal plants which are widely used in traditional systems of medicine[2]. Moringa oleifera Lam. Commonly known as hourse-radish or drumstick tree in English, belongs to family Moringaceae. It is a small sized tree, which is native to South Asia, Africa and Arabia and used as traditional medicine in many tropical and

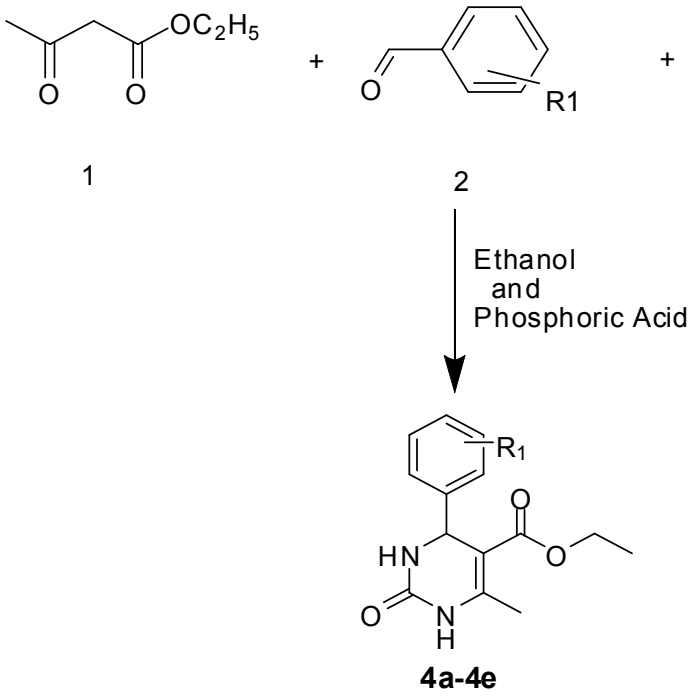
subtropical countries[3]. It is a dediduous tree growing rapidly even in poor soils, well adapted to droughts and able to reach up to 15 m in height. It is one of the 14 species of genus Moringa, which is native to India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America[4] The Flowers and the fruits appear twice each year, and seeds or cuttings are used to propagate the tree.

The leaf extracts of M. oleifera have been reported to exhibit antioxidant activity due to abundant phenolic acids and flavonoids[6]. The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation[7]. Flowes of M. oleifera are rich in calcium, potassium and antioxidants and are used in human diet, mainly in the Philippines[8]. As we learn that the antimicrobial agents are employed subdivision into different groups is possible. Subdivision can based upon the group of microorganisms affected like antibacterial, antifungal, antiprotozoal, antiviral and antiplastic chemotherapeutic agents, all more or less specific for the treatments of disease caused by specific pathogenic agents [9-10]. Inflammation is the means by which the body deals with insult and injury.Result may be caused: mechanically (eg. By pressure or foreign bodies) chemically (eg. By toxins, acidity, alkalinity) physically (eg. By temperature, by internal processes (eg.Uremia) and Fully understood communication between cellular and humoral elements[9]. Inflammation rids the body of the foreign matter and disposes of damaged cells, and initiates wound healing inflammation is controlled by mast cells that are in close proximity to autonomic nerves. Various molecule have been isolated from the plants which have been proven very effective in such condition. Drugs which are in use presently for the management if pain and inflammatory conditions are either narcotics.Eg. Opiods or Nannarcotics, salicylates and corticosteroids, hydrocortisone.

Materials and methods:

Preparation of 5-Ethoxycarbonyl-4-(substituted phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-ones

A mixture of – ethylacetoacetate (1) (10mmoles), substituted aldehyde (2) (10mmoles), urea (3) (30 mmoles) and phosphoric acid (3.54 mmoles), in presence of ethanol (5ml), urea mixed thoroughly and the reaction mixture was stirrer for 2 hours. After completion of reaction, the reaction mixture was poured on the crushed ice (10g) and stirred it, the solid product were filtered, washed with pet ether and subsequently dried it. The product were recrystallized from ethanol to afford pure 5-Ethoxycarbonyl-4-(substituted phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-ones (4a-4e).



4a-4e

Scheme 1 :
5-ethoxycarbonyl-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin-2(1H)-one

Where R_1 =a) –H , b) 4-OCH_3 , c) 4-NO_2 , d) 4-Br , e) 4-Cl

Analgesic activity

1. Preparation of Extract - The noninfected and matured stem bark of Oroxylumindicum(Family : Bignoniaceae) was collected from Champjai (23.456 ^{0} N Latitude and 93.329 ^{0} E Longitude), Mizoram, India, during the month of January. The plant was identified by the Department of Horticulture and Aromatic and Medicinal Plants , Mizoram University , Aizawl, India. The bark in clean and hygienic conditions. The dried bark was powdered in an electrical grinder at room temperature. The stem bark powder of O. indicum was sequentially extracted in petroleum ether, chloroform ,ethanol , and distilled water according to increase in polarity using a Soxhlet apparatus until the solvents became colourless[116]. The ethanol extract was concentrated using rotary evaporator and stored at -70^{0}C until further use . Henceforth the ethanol extract of O.indicum will be referred to as OIE throughout the paper.

2. Animal Care and Handling - The animal care handling were carried out according to the guidelines issued by the World Health Organization , Geneva, Switzerland , and INSA (Indian National Science Academy , New Delhi , India) . Usually , 6 to 8 week - old healthy male Swiss albino mice weighing 30-35 g were selected from an inbred colony maintained under the controlled conditions of temperature (25 ± 2^{0}C) and humidity (55-60%) with 12 hours of light and dark cycle , respectively. The animals had free access to standard rodent diet and water. All animals were housed in a sterile polypropylene cage containing paddy husk (procured locally) as bedding material. The animals had free access to standard rodent diet and water. All animal experiments were

carried out according to NIH and Indian National Science Academy, New Delhi, India guidelines. The study was approved by the Institutional Animal Ethics Committee of the Mizoram University , Aizawl , Mizoram: India vide letter number MZU/IAEC/4503.

3. Experimental - The anti - inflammatory and analgesic activities were determined by dividing the animals into the following groups.

3.1 SPS Group- The animals of this group did not receive any treatment except the sterile physiological saline (SPS) .

3.2 DIF Group- The animals of this group were injected with 20 mg/kg b. wt. of diclofenac sodium intraperitoneally.

3.3 OIE Group- The animals of this group were administered with 250 and 300 mg/kg b. wt. of ethanol extract of Oroxylumindicum intraperitoneally.

The analgesic activities were determined 30 minutes after the administration of SPS or diclofenac (DIF) or ethanol extract of Oroxylumindicum.

The analgesic activity of OIE was determined by carrying out the following tests.

a)Hot Plate Test - A separate experiment was conducted to determine analgesic activity of OIE by the hot plate as described earlier [117], where the grouping and other conditions were essentially similar to those described above . The hotplate contained metallic surface (diameter 20 and 10 cm high) and its temperature was set at 55^0C. Briefly, each mouse also acted as its own control . The time taken to lick the fore paws or jumping was recorded. Untreated animals exhibiting latency of 5-20 s were selected . The latency period for all groups was recorded thirty minutes after administration . Usually 10 mice were used for each group.

The percent inhibition was calculated as follows: (Posttreatment latency (s) – Pretreatment latency (s)) x 100/Pre- treatment latency (s)

b) Acetic Acid Induced Writhing Test - A separate experiment was performed to evaluate the analgesic activity by acetic acid induced writhing test, which was carried out as described earlier [118]. The grouping and other conditions were essentially similar to those described earlier . The mice were administered intraperitoneally with 0.7% v/v acetic acid (volume of acetic acid did not exceed 10uL/g b. wt.) . Immediately after acetic acid administration , the mice were individually placed into glass beakers and five min was allowed to elapse . The number of writhes produced in these animals was counted up to 30 min. For scoring purposes , a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Usually 10 mice were used for each group.

Inhibition of writhing (%) was calculated as follows =
Control – Treated/Control x 100

c)Tail Immersion Test- A separate experiment was conducted to evaluate the analgesic activity of Sonapatha ethanol extract by tail immersion test according to the procedure described else- where [119] . The grouping and other conditions were essentially similar to those described above in Section. The tail immersion test was carried out in a hot water bath set at a temperature of 55 ± 0.5^0 C, where 3 cm of animal tail was immersed into the hot water and tail withdrawal reaction was recorded as time in seconds in all groups using a digital stopwatch. A minimum of two observations were collected for each animal in control group, immediately and 10 min after the initial reading. The withdrawal test was carried out in the treatment groups periodically at 0, 0.5, 1, 2, 3, 4, and 6 hours after administration of OIE, or diclofenac. Usually 10 mice were used for each group.

Antioxidant

Ferric Reducing Antioxidant Power Assay

Reducing power assay method for determination of antioxidant activity/potential of samples

Procedure

Different concentrations of the drug (10-50ug/ml) was added to 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricynide [$K_3Fe(CN)6$] solution. The reaction mixture was vortexed well and then incubated at 50^0C for 20 min using vortex shaker. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid was addwd to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The coloured solution was red at 700 nm against the blank with reference to standaed using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard.

The percentage of radical scavenging (%) was calculated by the following formula:

$$\textbf{\% Free radical scavenging activity} = A_c - A_s / A_c \times 100$$

Where , A_c = Absorbance of control at 720 nm

A_s = Absorbance of sample

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

Anti-Fungal

The medium used for the study of antimicrobial activity of the newly synthesized compounds was Hi-Media Laboratories Pvt. Limited, India having following composition.

1. Nutrient Agar Medium (pH= 6.9-7.1)

Composition :- Peptone :5.0gm
Yeast extract :1.5gm
Beef extract :1.5gm
Sodium Chloride :5.0gm
Agar :5.0
Distilled Water :1000.0ml

2. Test Organism / Inoculum

Use four or five colonies of a pure culture to avoid selecting a typical variant or resistant clone. Prepare the incoculum by emulsifying overnight colonies from an agar medium, by diluting an overnight broth culture or by diluting a late log phase broth culture. The organism should be diluted in distilled water to a concentration of 10^7cfu/ml. A 0.5 McFarland standard gives a density equivalent to approximately 10^8cfu/ml. Inocula should be used promptly to avoid subsequent changes in their density. Plates should be inoculated within 30 minutes of standarising the inoculums.

3. Well

6 mm diameter well is formed in the middle of the solidified media for the inoculation of the test samples.

Procedure

i) Pick up a well isolated colony of the test organism and inoculate it in a nutrient broth. Incubate the broth at 37^0C 18 hrs. This young culture is to be used as incocular.

ii) Inoculate 0.5 ml of culture in sterile melted top agar previously cooled to 50 0C. Mix the top agar well and pour it evenly on the surface of sterile nutrient agar kept on the level surface. Allow the agar to solidify without disturbing it.

iii) When the top agar has solidified the well is made aseptically in the centre of the plate.
iv) Allow the plate to stand at room temperature or at refrigerator temperature for 30 minutes to permit the diffusion of antibiotics, before allowing the growth of the organisms.
v) Incubate the plate at 37 ^{0}C for overnight.
vi) After incubation period, measure the zone of inhibition. (zone diameter in mm).
vii) Interpret the results of the zone size, as sensitive, moderately sensitive and resistant, by comparing it with the interpretative chart provided with the multidisc.

The zone of inhibition observed around the well after respective incubation was measured in mm by using antibiotic zone reader. Standard used are Streptomycin and Penicillin.

Anti-inflammatory activity

Procedure

Different concentrations of the drug (10-50ug/ml) was added to 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricynide [$K_3Fe(CN)6$] solution. The reaction mixture was vortexed well and then incubated at 50^{0}C for 20 min using vortex shaker. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid was addwd to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The coloured solution was red at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard.

Percentage inhibition = 100 - [(A1 – A2)/ A0] × 100

Where, A1= Absorbance of test sample
A2 = Absorbance of productcontrol
A0= Absorbance of the positive control
Method used : Inhibition of Albumin Denaturation
Formula used:
% Inhibition = (Absorbance of control – Absorbance of sample /Absorbance of control × **100**

Result and Discussion

Analgesic Activity

The result of analgesic activities are presented in following Tables

Table 1Effect of Oroxylumindicum on the analgesic activity in mice by hot plate test

Treatment	Dose (mg/kg b.wt.)	Mean ± SEM		Increase in latency period (%)
		Pertreatment reaction latency (s)	Posttreatment reaction latency (s)	
Control	**0**	**7.60 ± 0.58**	**7.68 ± 0.50**	**0 %**
OIE	**300**	**8.00 ± 0.32**	**13.27 ± 1.14**	**62.9 %**
OIE	**250**	**7.60 ± 0.89**	**11.66 ± 1.10**	**52.66 %**
Diclofenac	**20**	**7.60 ± 0.55**	**13.45 ± 0.89**	**76.36 %**

N = 10 , P < 0.05 When Compared to SPS treated Control.

Table 2
Effect of ethanol extract of oroxylumindicum on xylene- induced ear edema in mice.

Treatment	Dose (mg/kg. b.wt)	Mean increase in ear weight (mg) ± SEM	% Inhibition
Control	0	14.08 ± 0.62	---
OIE	300	4.05 ± 0.29	71.42 **%**
OIE	250	4.48 ± 0.91	68.15 **%**
Diclofenac	20	6.62 ± 0.50	52.75 **%**

X = 10, P < 0.05 When compared to SPS treated Control

Table 3

Treatment	Dose (mg/kgb.wt.)	Mean ± SEM Number of writhes	Percentage inhibition of writhing (%)
Control	0	67.2 ± 2.16	0 **%**
OIE	300	21.8 ± 1.74	69.58 **%**
OIE	250	23.5 ± 2.12	67.01 **%**
Diclofenac	20	11.8 ± 1.74	84.68 **%**

Antioxidant Activity
Absorbance should increses with increase in concentration. Results confirm the reducing power (as indicated by absorbance at 700 nm) of samples which increased with increasing concentration. Higher value absorbance of the reaction mixture indicated greater reducing power. The reducing power was found to be in order of Br>NO_2.

Table 1 Ferric Reducing Antioxidant Power assay
Standard Observation - (Ascorbic acid) (720nm)

Concentration(ug/ml)	Absorbance 1	Absorbance 2	Absorbance 3
10ug/ml	0.002	0.006	0.061
20ug/ml	0.395	0.251	0.029
30ug/ml	0.941	0.775	0.499
40ug/ml	1.451	1.350	1.036
50ug/ml	2.000	2.000	2.000

Control Absorbance - 0.812 (720nm)
Sample Observation- (720nm)
Formula –
Scavenged (%) = (A_{cont}) - (A_{test}) /(A_{cont}) ×100

Table2 (% Scavenged of DPPH free radical)

Sample name	Concentration	Absorbance	%Scavenged
4a (H)	a) 30ug/ml b) 50ug/ml	0.121 0.305	72.1 %
4b (OCH_3)	c) 30ug/ml d) 50ug/ml	0.213 0.504	68.2 %
4c (NO_2)	a) 30ug/ml 50ug/ml	0.068 0.463	62.9%
4d (Br)	e) 30ug/ml f) 50ug/ml	0.171 0.629	17.1%
4e (Cl)	a) 30ug/ml b) 50ug/ml	0.058 0.102	21.2%

Antifungal Activity

Table 1 Antimicrobial activity test of 5-Ethoxycarbonyl-4-(substituted phenyl)-6-methyl-3,4- dihydropyrimidin-2(1H)-one. (4a-4e)

Compound	Antifungal	
	A. niger	***T. viride***
4a (H)	R	17
4b (p-OCH_3)	22	16
4c (p-NO_2)	---	15
4d (p-Br)	18	R
4e (p-Cl)	17	14
Streptomycin	15	17
Penicillin	18	16

(Diameter of inhibition zone in mm)

In Table 1

R : Resistant (10.0 mm and below) , S : Sensitive (10.0 mm and above), Slightly Sensitive : (10.0 mm above to 15.0 mm) , Moderately Sensitive : (15.0 mm above to 20.0 mm), Highly Sensitive : (20.0 mm above).

Anti-inflammatory Activity

Inhibition of protein denaturation

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakat et al. with slight modification. 500 uL of 1% albumin was added to 100 uL of sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51^0C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using

$$\textbf{Percentage inhibition} = 100 - [(A1 - A2)/ A0] \times 100$$

Where, A1= Absorbance of test sample

A2 = Absorbance of productcontrol

A0= Absorbance of the positive control
Method used : Inhibition of Albumin Denaturation

Formula used:
% Inhibition = (Absorbance of control – Absorbance of sample /Absorbance of control × **100**

Experimental -Study report of in vitro anti-inflammatory activity
Table 1 (% Inhibition of Protein Denaturation)

% Inhibition of Protein Denaturation						
Concentration µg/ml	**4a (H)**	**4b (OCH_3)**	**4c (NO_2)**	**4d (Br)**	**4e (Cl)**	**Std.(Diclofenac sodium)**
50 µg/ml	50 %	52 %	56 %	58 %	54 %	71 %
100 µg/ml	57 %	63 %	78 %	75 %	71 %	79 %
200 µg/ml	61 %	69 %	85 %	86%	82 %	86 %
400 µg/ml	93 %	78 %	92 %	89%	93 %	89 %
800 µg/ml	96 %	93 %	98 %	97 %	96 %	96 %

Method used : Inhibition Of Albumin Denaturation
Formula used:
% Inhibition = (absorbance of control – absorbance of sample/absorbance of control) x 100
Inhibition of Albumin denaturation method

Discussion:
Antifungal Activity : The compound synthesized this were Screened for their antimicrobial activity (Table 1). Compound 4b against the organism A. nigar were found to be highly sensitive. compounds 4d, 4e against A. nigar, 4a, 4b, 4c against T. viridewere found to be moderately sensitive. 4e against T. viride were found to be slightly sensitive ,where as compound 4a against A. nigar and 4d against T. viride were found to be Resistant.
Analgesic Activity : The analgesic activity of Oroxylumindicum was studied by the hot plate, tail immersion, and acetic acid tests, which are standard procedures to evaluate central and peripheral nervous system acting acid tests, which are standard procedures to evaluate central and peripheral nervous system acting analgesics [16-17]. The acetic acid is known to trigger the production of noxious substances within the peritoneum resulting in writhing response [16]. It is a simple, rapid, and reliable model and especially suitable to evaluate peripheral type of analgesic action of any drug [18]. The administration of Oroxylumindicum extract showed a significant analgesic activity indicating that it has some analgesic effect on both the central and peripheral nervous systems as indicated by reduced pain by hot plate method and suppression of acetic acid induced writhing .Several plants extracts including Adhatodavasica, Acacia hydaspica, Bossellia serrate, Glaucium grandiflorum, and Landolphiaowariensis have shown analgesic activity in vivo method[19-23].The tail immersion test has been used as a standard procedure to study the analgesic activity of pharmacological agents [24-25],which was originally devised by [26]. The increase in the tail withdrawal latency

is good measure of analgesia induced by any chemical agent. Treatment of mice with Oroxylumindicumextract increased tail withdrawal latency confirming its analgesic effects .

Antioxidant activity -Absorbance should increses with increase in concentration. Results confirm the reducing power (as indicated by absorbance at 700 nm) of samples which increased with increasing concentration. Higher value absorbance of the reaction mixture indicated greater reducing power. The reducing power was found to be in order of Br >NO_2.

Anti-inflammatory Activity-Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakat et al. with slight modification. Percentage of inhibition increses as the concentration increses.

Conclusion:

From the present investigation, it can be found that, some compounds exhibit strong activity against selected microbial species and some compounds show weak to moderate activity.
Also the result of anti inflammatory studies showed that the inhibition of albumin denaturation at the highest concentration of 1000µg/ml and membrane stabilization at the highestconcentration of 1600 µg/ml confirms the 4c &4d are potent inhibitor of acute and chronic inflammation.

A detailed research on different pharmacological activities can be carried out such as Hepatoprotective activity, analgesic activity and anti-inflammatory activity. Isolation of more active constituents possessing anti-inflammatory can be assessed.

Acknowledgements: The Authors are very thankful to the Department of Chemistry G.V.I.S.H. Amravati & G. S. Science, Art's & Commerce College, Khamgaon, for providing the necessary facilities in the Laboratory and also to Govt. Pharmacy College Amravati for anti-oxidant activity & Anti-inflammatory & CDRI, Lukhnow for providing the Spectral analysis.

References:

1) World Health Organuzation. Regulatory situation of herbal medicine. A worldwide review. Geneva : WHO, **1998**. [online] Available From: www.who.int/medicinedocs/pdf/whozip57e/whozip57e.pdf. [Accessed on 15 May, 2013]
2) Ghazanfar SA, Al Sabahi AMA. Medicinal plants of northern and central Oman. Econ Bot **1993**; 47: 89-98.
3) Moya B, Masika PJ, Hugo A, Muchenje V. Nutritional characterization of Moringa (Moringa oleifera Lam.)leaves. Afr J Biotech **2011**; 10(60): 12925-12933.
4) Muhl QE, Du Toit ES, Robbertse PJ. Moringa oleifera (horseradish tree) leaf adaptation to temperature regimes. Int J Agric Biol **2011**; 13: 1021-1024.
5) Verma AR, Vijaykumar M, Mathela CS, Rao CV. In-vitro and in-vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem Toxicol**2009**; 47: 2196-2201.
6) Atawodi SE, Atawoli JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, et al. J Med Food **2010**; 13(3): 710-716.
7) Manaheji H, Jafari S, Zharinghalam J, Rezazadeh S, Taghizadfarid R. Analgesic effect of methanolic extract of the leaf or roots of Moringa oleifera on complete Freund's adjuvant induced arthritis in rats. J Chinese Int Med **2011**; 9(2): 216-222.

8) Pontul EV, Carvalho BEA, Bezerra RS, Coelho LCB, Napoleao TH, Paiva PMC. Caseinolytic and milk-clotting activities from Moringa oleifera flowers. Food Chem **2012**; 135(3): 1848-1854.
9) Cruickshank R., Duguid J.P., Maramion B.P. ,. Swain R.H.A., "Medicinal Microbiology,12thEdn." ,**1975**, 2.
10) FuerstR. ,"Microbiology in Health and Disease, 5thEdn.," W.B. Saunder Co., Tokyo, Igaku-Shoin/Saunders , **1983**.
11) Sofowora, A., Medicinal plants and traditional medicines in Africa. 2nd Edition. Karthala Ibadam Nigeria **1993**.
12) Fu, N.Y.; Pang, M. L.; Yuan, Y. F.; Wang, J. T. Chin. Chem. Lett., **2002**, 13, 921.
13) Saleem, T.K., Azeem, A.K., Dilip, C., Sankar,C., Prasanth, N.V and Duraisami,R. Anti inflammatory activity of leaf extracts of Gendarussa vulgaris Nees. Asian Pacific Journal of Tropical Biomedicine, **2011**,147-149.
14) Chandra,s., Chatterjee,P., Dey,P and Bhattacharya,s, Evaluation of anti inflammatory effect of ashwagandha: A preliminary study in vitro. Pharmacognosy Journal, **2012**,4(29), 47-49.
15) Sangeetha, M., Kousalya, K., Lavanya, R., Sowmiya, S., Chamundeeswari, D., and Maheswara, U.C,Anti inflammatory and anti arthritic activity of leaves of Cleodendroninerme. Research jouranal of Pharmaceutical biological and chemical science **2011**, 2(1), 822-827.
16) Bartolini A., Galli A., Ghelardini C., et. Antinociception induced by systematic administration of local anaesthetics depends on a central cholinergic mechanism. British Journal of Pharmacology, **1987**, 92(4),711 -721. Doi: 10.111/j.1476-5381.1987.tb 11375.x. [PMC free article] [PubMed] [CrossRef]
17) Hiruma-Lima C. A., Gracioso J. S., Bighetti E. J. B., GermonsenRobineou L., Souza Brito A. R. M. The juice of fresh leaves of Boerhaaviadiffusa L. (Nyctaginaceae) markedly reduses pain in mice. Journal of Ethnopharmacology, **2000** ,71(1-2),21-27. Doi: 10. 1016/s0378-8741(oo)00178-1. [PubMed] [CrossRef]
18) Shinde U.A., Phadke A.S., Nair A.M., Mungantiwar A.A., Dikshit V. J., Saraf M. N. Studies on the anti-inflammatory and analgesic activity of cedrusdeodara (Roxb). Loud. Wood oil. Jouranal of Ethnopharmacology, **1999** ,65(1),21-27. Doi: 10.1016/s0378-8741(98)00150-0. [PubMed] [CrossRef]
19) Owoyele B. V., Olaleye S. B., Oke J. M., Elegbe R. A. Anti-inflammatory and analgesic activities of leaf extracts of LandolphiaOwariencsis. African Journal of Biomedicasl Research, **2010**, 4(3),131-133. Doi, 10.4314/ajbr.v4i3.53896. [CrossRef]
20) Morteza-Semnani K., Saeedi M., Hamidian M. Anti-inflammatory and analgesic activity of the topical prepration of Glaucium grandiflorum. Fitoterapia, **2004**, 75(2), 123-129. Doi:10.1016/j.fitote.2003.12.007. [PubMed] [CrossRef]
21) Sharma A., Bhatia S., Kharya M.D., et al. Anti-inflammatory and analgesic activity of different fractions of Boswellia serrata. International Journal of Phytomedicine, **2010**, 2(1), 94-99. Doi: 10.5138/ijpm.2010.0975.0185.02015. CrossRef]
22) Mulla W. A., More S. D., Jamge S.B., Pawar A. M., Kazi M. S., Varde M. R. Evaluation of anti-inflammatory and analgesic activities of ethanolic extract of roots Adhatodavasica Linn. International Journal of Pharm Tech Research, **2010** ,2(2), 1364-1368.
23) Afsar T., Khan M. R., Razak S., Ullah S., Mirza B. Antipyretic, anti-inflammatory and analgesic activity of Acacia hydaspica R. Parker and its phytochemical analysis. BMC Comlementary and Alternative Medicine, **2015**,15, article 136 doi: 10.1186/s12906-015-0658-8. [PMC free article] [PubMed] [CrossRef]

24) Luttinger D. Determination of antinociceptive efficacy of drugs in mice using different water tempratures in a tail-immersion test. Journal of Pharmacological Methods, **1985**, 13(4):351-357. Doi: 10.1016/0160-5402 (85)90017-8 [PubMed] [CrossRef]
25) Dyksrea L. A., Gmerek D.E., Winger G., Woods J.H. Kappa opioids in rhesus monkeys. Diuresis, sedation, analgesia and discriminative stimulus effects. Journal of Pharmacology and Experimental Therapeutics, **1987**, 242(20,413-420. [PubMed]
26) Janssen P. A., Niemgeers C. J., Dony J. G. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawl reflex in rats. Arzneimittel- Forschung. **1963**,13,502-507 [PubMed].

Received on March 31, 2019.