ELAEOCARPUS SERRATUS L. EXHIBITS POTENTIAL ANALGESIC AND ANTIDIARRHEAL ACTIVITIES IN MICE MODEL

A.A.H. Pinkey1, *Z.I. Khan2, M.A. Taher1, M.A. Soma1

1 – DEPARTMENT OF PHARMACY, STATE UNIVERSITY OF BANGLADESH, DHAKA, BANGLADESH
2 – DEPARTMENT OF HEALTH TECHNOLOGY AND INFORMATICS, THE HONG KONG POLYTECHNIC UNIVERSITY, HONG KONG, CHINA

Background. Elaeocarpus serratus L. (Family: Elaeocarpaceae) is a tropical fruit tree, traditionally used in the treatments of poisoning, diarrhea, arthritis, and other diseases.

Objectives. The current study was performed to conduct the analgesic, antidiarrheal, and hypoglycemic activity of E. serratus in mice model using methanolic bark crude extract.

Methods. To assess the peripheral and central analgesic activity, the acetic acid-induced writhing and tail immersion methods were respectively used. The castor-oil mediated antidiarrheal method was used to assess the antidiarrheal activity whereas, the tail tipping technique was conducted to determine the hypoglycemic activity of the plant extract.

Results. In the peripheral analgesic assay, the methanolic bark crude extract of E. serratus significantly inhibits the number of writing 69.77% (200 mg/kg) and 73.26% (400 mg/kg) respectively (p<0.05) which was strongly comparable with standard NSAID drug diclofenac sodium 75.58% (p<0.05). Similarly, it shown a significant tail flicking response for 30 minutes, 60 minutes and 90 minutes of central analgesic activity assay. In antidiarrheal activity assay, the E. serratus substantially reduced the number of diarrheal feces 64.26% (200 mg/kg, p<0.05) and 78.57% (400 mg/kg, p<0.05) which was also comparable with the positive control loperamide. The hypoglycemic activity of E. serratus extract was not convincing.

Conclusions. Our investigation demonstrated the significant analgesic and antidiarrheal activities of methanolic bark extract of E. serratus (200 and 400 mg/kg) in mice model.

KEYWORDS: Elaeocarpus serratus; analgesic activity; antidiarrheal activity; hypoglycemic activity.

Introduction

Medicinal plants or natural drugs are traditionally and historically used around the globe by human beings for curing various ailments. The plant-derived natural drugs are widely accepted to all due to their diverse pharmacological activities, reduced toxicity, cost-effective, availability for drug discovery, and application to the chemical biology [1, 2]. Although, the incessant investigation is being carried out to screen potential pharmacological activities of natural products but the numbers are very limited considering all medicinal plants distributed throughout the world [3]. So far, a considerable number of experimental research have been reported the use of natural products as an antioxidant agent, blood glucose-lowering agent, antimicrobial agent, central nervous system (CNS) stimulating agent, anti-diarrheal agent, anti-helminthic agent, anti-inflammatory agent, and anti-cancer agent [4]. By considering the previous studies, we explored the pharmacological activities of Elaeocarpus serratus L. (E. serratus) in a number of biological uses.

E. serratus (English name: Rosary nut, Ceylon olive, Bengali name: Jalpai) belongs to the family Elaeocarpaceae, a tropical fruit tree grown up to 18 meters tall, distributed in evergreen forests, and sometimes also cultivated for its edible fruit and medicinal applications [5, 6]. It is mostly found in the Indian subcontinent regions including India, Bangladesh, Pakistan, Sri Lanka and Nepal. However, it is also found in Indo-China regions including, Myanmar, Indonesia, Thailand and Malaysia [5]. The E. serratus is a plant having both nutritional and medicinal values. For instance, the GC-MS analysis revealed that the plant contains numerous compounds including fatty acid, alcohol, aldehyde, hydrocarbons and alkenes which are biologically active [7]. In
addition, the leaf of E. Serratus contains alkaloids, flavonoids, and glycosides (eg. anthraquinone) [7]. Moreover, a list of bioactive compounds also contained in E. serratus such as myricitrin, mearnssetin 3-O-β-D-glucoside, mearnsitrin, and tamarixetin 3-O-α-L-rhamnopyranoside where, myricitrin is an established potential antioxidant [8]. Historically, leaves of E. serratus extracts are used for the treatments of arthritis and various poisoning [9]. Equally, appetite, diarrhea, dysentery and other neuro-motors related diseases are commonly treated with fruits or fruit extracts [6, 10]. Moreover, the previous studies also reported that the leaf, bark and fruit of E. serratus have antimicrobial and antifungal activities [11, 12]. For all we know, there is no scientific report conducted on analgesic, hypoglycemic, and anti-diarrheal properties of E. serratus yet. Therefore, our main objective was to assess the analgesic, hypoglycemic, and antidiarrheal activity of methanolic bark crude extract of E. serratus in mice model.

Methods
Collection and extraction of plant
In February 2018, the bark of E. Serratus was acquired from Chandpur, Bangladesh. The collection of bark samples was verified by Bangladesh National Herbarium (BDNH), Dhaka, Bangladesh. An herbarium specimen number (DACB-31155) was provided and preserved for their further reference. The barks were cleaned and cut into small pieces to accelerate the drying process. Then the sun-dried fragments were crushed to a fine powder. About 400 g of powder was put in a flat bottom amber sterile glass container and socked with 1.5L methanol for two weeks. Continuous shaking and stirring were maintained over time. Afterward, the entire mixture was filtered with cotton and repeated second filtration with Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was then kept for a week to monitor any suffering or distress and fasted overnight prior to the experiments. The animal experiments were conducted according to the Ethics Committee of State University of Bangladesh (SUB), Dhaka, Bangladesh.

Drug treatments and chemical reagents
Diclofenac sodium, glibenclamide, and loperamide hydrochloride were purchased from Beximco Pharmaceuticals Ltd (Bangladesh). Phenobarbitone sodium and morphine sulphate were supplied by Incepta Pharmaceuticals Ltd (Bangladesh), and Beacon Pharmaceuticals Ltd (Bangladesh). Tween 80, normal saline (0.9% NaCl) and castor oil were kindly given by BDH Chemicals Ltd (United Kingdom). The remaining chemicals and reagents were purchased from Sigma-Aldrich (Munich, Germany).

Peripheral analgesic activity
The acetic acid-mediated writhing method by Kaushik, D., et al. 2012 was copied to assess the peripheral analgesic activity of the E. serratus crude extract [13]. The intraperitoneal acetic acid injection was given to all mice with a view to exhort the abdominal pain followed by writhing in mice. The potentialities of the test samples were measured by calculating their competency in the reduction of writhing numbers. Test group (ES-I and ES-II) were orally administered, containing the doses of 200- and 400 mg/kg of body weight (b.w.) (ES-I) and 400 mg/kg b.w. (ES-II). Mice were remarkably observed for a week to monitor any suffering or distress and fasted overnight prior to the experiments. The writhing cases were carefully observed and documented for 10 minutes after giving 10 minutes resting period. The acetic acid-induced pain reduction was calculated by using the following equation:
Central analgesic activity

Pizziketti et al., 1985 described the tail-flick method was implemented to assess the central analgesic activity of E. serratus crude extract in mice [15]. In this method, the mice were orally given a different dose of drugs and E. serratus, and the tips of the mice tails were submerged in a constant radiant heat source (hot water bath at 55±0.5 °C). The reaction time (mice tail deflects from the heating source) of each mouse was recorded using a stopwatch. To prevent the damage of tail, we maintained a cut off period of 15 seconds. Similar to the peripheral analgesic study, the NC group orally received 1% tween-80 in saline, and the PC group was subcutaneously injected with morphine (2 mg/kg b.w.) [16]. The ES-I and ES-II were prescribed orally to the test groups of mice. The tail-flick reaction was counted and recorded in 0 minutes, 30 minutes, 60 minutes, 90 minutes after administration of the test samples. The following equation was used to measure the pain inhibition percentage (PIP).

\[
\text{PIP} = \frac{\text{Mean latency of treatment} - \text{Mean latency of control}}{\text{Mean latency of control}} \times 100\%
\]

Hypoglycemic activity

The tail tipping technique according to the method described by Durschlag et al., 1996 was repeated to assess the hypoglycemic activity of test samples in mice model [17]. Here, the NC group was treated with 1% tween-80 (0.1 ml/10 mg b.w.), and PC group treated with glibenclamide (5 mg/kg b.w.) whereas, Group-III and Group-IV were treated with ES-I and ES-II respectively. All treatments were applied orally [14]. To accelerate the blood sugar level of mice, a 10% glucose solution was orally given to all mice after an hour resting period at dose 2 g/kg b.w. Blood is withdrawn from the tail tip and blood sugar was measured and recorded by using a glucometer (Bioland G-423 S) in 0 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes respectively.

\[
\text{Percentage inhibition} = \frac{\text{Mean defecation of control} - \text{Mean defecation of test sample or standard}}{\text{Mean defecation of control}} \times 100\%
\]

Statistical analysis

The values are represented here are set of mean ± standard error of mean (M±SEM). All the calculation was performed using student t-test or one way ANOVA followed by Dunnett’s test to determine the statistically significant differences between the groups. A p-value < 0.05 was considered statistically significant.

Results

The peripheral analgesic activity of E. serratus crude extract is demonstrated in Table 1. A significant reductions of abdominal muscle contractions caused by the administration of 0.1 ml acetic acid were exhibited in both experimental groups where ES-II showed higher writhing inhibition and was close to the PC group. Our results indicated that the E serratus bark crude extracts significantly inhibit the number of writhing 69.77% and 73.26% at dose 200 and 400 mg/kg b.w. gradually whilst diclofenac sodium displayed 75.58% writhing inhibition.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC=diclofenac sodium, ES-I: E serratus crude extract-I, ES-II: E serratus crude extract-II. M1-4=mice 1 to 4 respectively. (n=4, *p<0.01)

The result of the tail-flick method to assess the central analgesic activity of E. serratus are
shown in table 2. Both experimental groups ES-I and ES-II increased the response time by 37.32% and 53.72% respectively in the initial 30 minutes of the experiment, whereas PC morphine increased by 85.24%. In addition, 82.76% (200 mg/kg b.w.), 98.94% (400 mg/kg b.w.) tail flicking response were recorded in 90 minutes of the experiments. The whole experiment followed a dose-dependent tail flicking response over the time.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC= morphine sulfate, ES-I: E. serratus crude extract-I, and ES-II: E. serratus crude extract-II. (n=4, *p<0.01)

The bark crude extract of E. serratus not displayed any significant blood glucose-lowering activity at doses 200 and 400-mg/kg b.w. However, the percent of blood sugar reducing activity was found to be followed in a dose-dependent manner. The results shown in table 3 indicated that the highest glucose lowering activity was displayed at dose 400 mg/kg b.w. relative to ES-I groups.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC= glibenclamide, ES-I: E. serratus crude extract-I, and ES-II: E. serratus crude extract-II. (n=4, *p<0.01)

The remarkable antidiarrheal activities were displayed by ES-I and ES-II in mice. The potential antidiarrheal activity of the E. serratus crude extract is shown in table 4. The ES-I and ES-II substantially reduced the number of castor oil-incited diarrheal feces by 64.29% and 78.57% compared to the NC. The highest diarrheal reduction was shown by PC group.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC= loperamide hydrochloride, ES-I: E. serratus crude extract-I,
and ES-II: *E. serratus* crude extract-II. (n=4, *p<0.01)

**Discussion**

Acetic acid may trigger the writhing reflex in experimental animals where visceral pain is generated through activation of pain receptors on the visceral surface and extreme secretion of histamine, prostaglandins, bradykinin and serotonin [20]. In the experimental animals, acetic acid induces visceral pain which is commonly treated with NSAID drugs or chemicals, such as phenyl quine (prostaglandin E2 inhibitor). In addition, the level of analgesia is measured by calculating the percent reduction of abdominal contraction by drugs or crude extract after intraperitoneal administration of acetic acid to mice. In this study, *E. serratus* extracts significantly reduced the sum of abdominal contraction of 69.77% and 73.26% by ES-I and ES-II compared to NC. Importantly, the results of peripheral analgesic activity by ES-I and ES-II were almost equal to the diclofenac treatment. Therefore, by considering our results, we assumed that *E. serratus* extract may be inhibited the synthesis or release of endogenous substances in mice to act its potential peripheral analgesic activity. However, further research may need to explore the exact mechanisms.

In the central analgesic assay, the relative promotion of tail-flicking response (in percent) was obtained from *E. serratus* extract in a dose and time-dependent manner. Although, the responses from *E. serratus* crude extracts were a bit of lower than the PC-morphine however, higher dose might be shown an equals or higher potentiality like morphine. Pizziketti, et al., 1985 demonstrated that the tail flicking response is mostly generated from spinal reflex caused by radiant heat source however it may involve higher neuronal complex signals. In general, the pain is centrally originated via a number of complex signaling such as opiate, dopaminergic, noradrenergic and serotonergic nervous systems [15]. Our results described that *E. serratus* displayed a significantly higher level of pain threshold activity at 200 and 400 mg/kg b.w. respectively in mice model. The core mechanisms may be associated with the receptor-bind inhibition of pain-related nervous system or through peripheral mechanisms involved prohibited prostaglandins, leukotrienes, and other endogenous substances release and synthesis which are key mediators of pain [21]. Our results might be followed the same mechanisms to exhibit the potential analgesic activity in mice model.

Our bark crude extract of *E. serratus* showed lack of blood glucose lowering activity. Notwithstanding, a considerable number of studies have concluded that plant extracts exhibit potential anti-hyperglycemic activity by accelerating or regenerating β cells or promoting the secretion of insulin [22, 23]. The hypoglycemic activity by the natural product may also associated with excessive insulin secretion from β cells or trigger the peripheral glucose consumption, or promote insulin-mediated blood sugar absorbing mechanisms [22-24].

Apart from this, the statistical evaluation revealed that both doses of *E. serratus* showed a significant dose-dependent anti-diarrheal activity in mice. The ricinoleic fatty acid or 12-hydroxy-9-cis-octadecenoic acid is an active metabolite of castor oil. This metabolic fatty acid enhanced peristaltic activity in the small intestine to trigger the permeability of mucosal electrolytes thus resulting diarrhea [25, 26]. Furthermore, ricinoleic fatty acid enhanced mucosal irritation and inflammation which contribute to the excessive endogenous prostaglandin secretion. Moreover, in castor oil-induced diarrheal mechanisms it involved a cascade of signaling including, intestinal Na+/K’-ATPase inhibition, adenylate cyclase activation or promotion cAMP-mediated platelet-activating factor secretion [25, 27].

In summary, the plant *E. serratus* contained several flavonoids, anthraquinone glycosides, fatty acid, alcohol, aldehyde, hydrocarbons algaloids, terpenoids, and steroids. [7, 8, 28]. The presence of glycosides, steroids, and flavonoids which exhibited potential analgesic, hypoglycemic and anti-diarrheal activities in many plants [29-31]. In the present study, we concluded that *E. serratus* extract may contain a variety of bioactive phytochemicals. After successful isolation and characterization of phytochemicals, it might be used as an analgesic, and as an antidiarrheal agent.

**Conclusion**

The bark extract of *E. serratus* exhibited potential peripheral and central analgesic activity, very mild hypoglycemic activity but effective antidiarrhoeal activity in mice model. Therefore, further investigations are needed to isolate and characterization of bioactive molecules present in this plant. Further research may open a new therapeutic agents in the treatments of various diseases.
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Conflict of Interests

Authors declare no conflict of interest.

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Author's Contributions

Asma Aul Husna Pinkey, Mohammad Abdullah Taher – conceptualization, methodology; Asma Aul Husna Pinkey, investigation, data curation, formal analysis, writing – original draft; Zahirul Islam Khan – data curation, formal analysis, writing – reviewing and editing; Mahfuza Afroz Soma – writing – reviewing and editing.

Преклінічні дослідження зенеболюючої та протипроносної дії Elaeocarpus serratus L. на миšíах

A.A.H. Pinkey1, *Z.I. Khan2, M.A. Taher1, M.A. Soma1

1 - DEPARTMENT OF PHARMACY, STATE UNIVERSITY OF BANGLADESH, DHAKA, BANGLADESH
2 - DEPARTMENT OF HEALTH TECHNOLOGY AND INFORMATICS, THE HONG KONG POLYTECHNIC UNIVERSITY, HONG KONG, CHINA

Вступ. Elaeocarpus serratus L. (родина Elaeocarpaceae) - тропічне плодове дерево, фрукти, кора та інші частини якого традиційно використовуються при лікуванні отруєнь, діареї, артриту та інших захворювань.

Мета – дослідити фармакологічну активність (зенеболювальну, протидіарейну та гіпоглікемічну дію) сухого метанольного екстракту кори E. serratus на миšíах.

Методи. Для експериментальної оцінки центрального та периферичного компонентів у механізмі зенеболювальної дії екстракту використовували метод оцінки больової реакції, що викликається хімічним подразненням – метод «сушоковокислих карчів», та метод теплового подразнення, суттєво що полягає в зануренні хвоста миші у гарячу воду (55±0.5°C). Для оцінки протипроносної активності використовували модель діареї, викликаної введенням рицинової олії, для визначення гіпоглікемічної активності екстракту використали метод Durschlag et al., 1996, забір крові проводили шляхом надрізів хвоста.

Результати. Встановлено, що застосування сухого метанольного екстракту кори E. serratus достовірно зменшує частоту розвитку корчів на 69,77% (200 мг/кг) та 73,26% (400 мг/кг) відповідно (р<0,05), що досягає рівня активності стандартного НПЗП диоксифенокси. Якій зменшує показник на 75,58% (р<0,05). Такі ж результати щодо частоти реакції хвоста піддослідних тварин протягом 30, 60 та 90 хвилин – показника центральної зенеболюючої активності екстракту. Щодо протипроносної активності, то E. Serratus зменшував частоту діареї на 64,26% (200 мг/кг, р<0,05) та 78,57% (400 мг/кг, р<0,05), що також досягало також ж ефективності, як і у групі позитивного контролю з лоперамідом. Щодо гіпоглікемічної активності екстракту E. serratus – отримані нами результати були неперевершені.

Висновок. Наše дослідження продемонструвало значну зенеболювальну та протидіарейну активність сухого метанольного екстракту кори E. serratus (200 та 400 мг/кг) на миšíах.

КЛЮЧОВІ СЛОВА: Elaeocarpus serratus; зенеболювальна активність; протипроносна активність; гіпоглікемічна активність

Information about the authors

Asma Aul Husna Pinkey, graduate student, State University of Bangladesh, Dhaka, Bangladesh
ORCID 0000-0002-6851-269X, e-mail: pinkeykhanam88@gmail.com

Md Zahirul Islam Khan, full-time PhD student, The Hong Kong Polytechnic University, Hong Kong, China
ORCID 0000-0001-7048-2613, e-mail: zahir.islamkhan@connect.polyu.hk

Mohammad Abdullah Taher, lecturer and coordinator, State University of Bangladesh, Dhaka, Bangladesh
ORCID 0000-0002-0701-470X, e-mail: taher@sub.edu.bd

Mahfuza Afroz Soma, lecturer and Student Counselor, State University of Bangladesh, Dhaka, Bangladesh
ORCID 0000-0003-2903-8822, e-mail: soma@sub.edu.bd
References

1. Saleh-e-In MM, Sultana N, Hossain MN, Hasan S, Islam MR. Pharmacological effects of the phytochemicals of Anethum sowa L. root extracts. BMC Complement Altern Med. 2016 Dec;16(1):464. DOI: https://doi.org/10.1186/s12906-016-1438-9
2. Khan MF, Khan ZI, Uddin MR, Rahman MS, Rashid MA. In vivo hypoglycemic and alloxan induced antidiabetic activity of Xeromphis uliginosa Retz. Afr J Pharm Pharmacol. 2015;9(11):363-6. DOI: https://doi.org/10.5897/AJPP2015.4293
3. Al-Snafi AE. Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants. Rigi Publication; 2015.
4. Van Wyk E, Wink M. Medicinal plants of the world. CABP; 2018. DOI: https://doi.org/10.1079/9781786393258.0000
5. Baruah PS, Deka K, Lakhar L, Sarma B, Borah SK, Tanti B. Habitat distribution modelling and reinforcement of Elaeocarpus serratus L.- A threatened tree species of Assam, India for improvement of its conservation status. Acta Ecologica Sinica. 2019;39(1):42-9.
6. de Lima FF, Breda CA, Cardoso CAL, Duarte MCT, Sanjinez-Argandoña EJ. Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of Elaeocarpus serratus fruit extract. Afr J Food Sci. 2019;13(1):30-7. DOI: https://doi.org/10.5897/AJFS2018.1760
7. Geetha D, Rajeswari M, Jayashree I. Chemical profiling of Elaeocarpus serratus L. by GC-MS. Asian Pac J Trop Biomed. 2013;3(12):985-7. DOI: https://doi.org/10.1016/j.cnab.2018.06.002
8. Jayasinghe L, Amarasinghe NR, Arundathie BS, Rupasinghe G, Jayatilake NAN, Fujimoto Y. Antioxidant flavonol glycosides from Elaeocarpus serratus (Elaeocarpaceae). Int J Pharm Sci Res. 2015;6(6):2649.
9. Hardainiyan S, Nandy BC, Kumar K. Elaeocarpus ganitrus (Rudraksha): a reservoir plant with their pharmacological effects. Int J Pharm Sci Rev. 2015;34:55-64.
10. Jayashree I, Geetha D, Rajeswari M. Evaluation of antimicrobial potential of Elaeocarpus serratus L. Int J Pharm Sci Res. 2014;5(8):3467-72.
11. Snah S, Sharath R, Aishwarya K, Samrat K, Vasundhara M, Radhika B. Screening of the antioxidant, antibacterial and cytotoxic activities of the methanolic extracts of Elaeocarpus ganitrus and Elaeocarpus serratus. Int Res J Innov Eng. 2015;1:1-11.
12. Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and anti-inflammatory activity of Pinus roxburghii Sarg. Adv PharmacoSci. 2012;2012:245431. doi:10.1155/2012/245431. DOI: https://doi.org/10.1155/2012/245431
13. Sharmin T, Rahman MS, Mohammadi H. Investigation of biological activities of the flowers of Lagerstroemia speciosa, the Jarul flower of Bangladesh. BMC Complement Altern Med. 2018;18(1):231. DOI: https://doi.org/10.1186/s12906-018-2286-6
14. Pizziketti R, Pressman N, Geller E, Cowan A, Adler M. Rat cold water tail-flick: a novel analgesic test that distinguishes opioid agonists from mixed agonist-antagonists. Eur J Pharmacol. 1985;119(1-2):23-9. DOI: https://doi.org/10.1016/0014-2999(85)00379-2
15. De Lima FF, Breda CA, Cardoso CAL, Duarte MCT, Sanjinez-Argandoña EJ. Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of Elaeocarpus serratus fruit extract. Afr J Food Sci. 2019;13(1):30-7.
16. Adeyemi OO, Okpo SO, Ogunji OO. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana Mill (Lauraceae). Fitoterapia. 2002;73(5):375-80. DOI: https://doi.org/10.1016/S0367-326X(02)00118-1
17. Dürschlag M, Würbel H, Stauffacher M, von Holst D. Repeated blood collection in the laboratory mouse by tail incision-modification of an old technique. Physiol Behav. 1996;60(6):1565-8. DOI: https://doi.org/10.1016/S0031-9384(96)00307-1
18. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J Ethnopharmacol. 2001;76(1):73-6. DOI: https://doi.org/10.1016/S0378-7841(00)00379-2
19. Pal A, Al Mahmud Z, Akter N, Islam S, Bachar SC. Evaluation of Antinociceptive, Antidiarrheal and Antimicrobial Activities of Leaf Extracts of Clerodendrum indicum. Phcog J. 2012;4(30):41-6. DOI: https://doi.org/10.5530/pj.2012.30.8
20. Padi SSV, Kulkarni SK. Minocycline prevents the development of neuropathic pain, but not acute pain: Possible anti-inflammatory and antioxidant mechanisms. Eur J Pharmacol. 2008;601(1):79-87. DOI: https://doi.org/10.1016/j.ejphar.2008.10.018
21. Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. Iran J Pharm Res. 2015;14(1):263-9.
22. Hannan J, Marenah L, Ali L, Rokeya B, Flatt P, Abdel-Wahab Y. Ocmium sanctum leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β-cells. J Endocrinol. 2006;189(1):127-36. DOI: https://doi.org/10.1677/joe.1.06615
23. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant Coriandrum sativum (coriander). Br J Nutr. 1999;81(3):203-9. DOI: https://doi.org/10.1017/S0007114599000392
24. Ota A, Ulrih NP. An overview of herbal products and secondary metabolites used for management of type two diabetes. Front Pharmocol. 2017;8:436.
   DOI: https://doi.org/10.3389/fphar.2017.00436
25. Iwao I, Terada Y. On the mechanism of diarrhea due to castor oil. Jpn J Pharmocol. 1962;12(2):137-45.
   DOI: https://doi.org/10.1254/jjp.12.137
26. Bright-Asare P, Binder HJ. Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. Gastroenterology. 1973;64(1):81-8.
   DOI: https://doi.org/10.1016/S0016-5085(73)80094-0
27. Tiruppathi C, Balasubramanian K, Hill P, Mathan V. Faecal free fatty acids in tropical sprue and their possible role in the production of diarrhoea by inhibition of ATPases. Gut. 1983;24(4):300-5.
   DOI: https://doi.org/10.1136/gut.24.4.300
28. Jayashree I, Geetha D, Rajeswari M. Evaluation of antimicrobial potential of Elaeocarpus serratus L. Int J Pharm Sci and Res. 2014;5(8):3467.
29. Zhang D-W, Cheng Y, Wang N-L, Zhang J-C, Yang M-S, Yao X-S. Effects of total flavonoids and flavonol glycosides from Epimedium koreanum Nakai on the proliferation and differentiation of primary osteoblasts. Phytomedicine. 2008;15(1-2):55-61.
   DOI: https://doi.org/10.1016/j.phymed.2007.04.002
30. Kajaria DK, Gangwar M, Sharma AK, Nath G, Tripathi Y, Tripathij, et al. Comparative evaluation of phenol and flavonoid content of polyherbal drugs. Pharmacologyonline. 2011;3:1365-73.
31. Arif M, Fareed S. Pharmacognostical studies and evaluation of total phenolic and flavonoid contents of traditionally utilized fruits of Solanum torvum Sw. Indian J Nat Prod Resour. 2011;2(2):218-24.

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