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Assessment of Phytochemical, Analgesic and Antioxidant Profile of *Melia azedarach* L. [Leaves] (Family-Meliaceae)

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This project is designed to evaluate the potential analgesic and antioxidant activity of *Melia azedarach* L. to judge the traditional uses of this medicinal plant. The ethanolic extract of the dried leaves of *Melia azedarach* L. (Family: Meliaceae) was assessed for its possible analgesic and antioxidant activity. Photochemical screening of the ethanolic extract revealed the presence of alkaloids, glycosides, tannins, Saponins and Flavonoids. In acetic acid induced writhing in mice, the ethanolic extract (250 and 500mg/kg) exhibit significant (P<0.001) inhibition of writhing reflex 45.45% and 67.05% respectively compared to standard Diclofenac Sodium 76.14% (P<0.001) at a dose 25mg/kg body weight. The ethanolic extract showed antioxidant activity. In qualitative antioxidant assay using 1, 1-diphenyl-2-picryl hydrazyl the extract showed free radical scavenging properties. In the quantitative assay, extract displayed free radical scavenging activity in the assay (IC50~95µg/mL) which is comparable to that of ascorbic acid (IC50 ~15µg/mL), a well-known standard antioxidant.

Keyword: *Melia azedarach* L., Meliaceae, Antioxidant activity, DPPH, Analgesic activity.

1. Introduction

Disease is as old as life and man has until the end of time been in look for the agents to treat diseases. Medicinal plants and related one have been in use for eradication of diseases and human suffering since ancient times. The development of the medicinal values of plants throughout the ages was accomplished principally through careful inspection, trial and error, and unintentional discovery. In this process, the human race, over the centuries, has created a vast heritage of knowledge and experience on medicinal plants in different cultures and civilizations. The major portion of the present day knowledge of the medicinal properties of plants is the sum total of some observations and experiences. Some of the wonder drugs of modern medicine have their roots in their early knowledge of medicinal plants. According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants [1]. Combining nature with science had been started since ancient era and now been practicing extensively. Nature has provided a complete reservoir of remedies to cure all aliments of mankind. *Melia azedarach* L. is a deciduous tree up to 45m tall, fluted shaft from 30-60 (Maximum 120) cm in diameter, with a spreading crown and sparingly branched limbs age. Skin smooth, greenish-brown when young, turning gray and cracked with age. The leaves are alternate, long 20-40, pinnate or sometimes three times pinnate twice cm. Leaflets 3-11, jagged and with a pungent odor when crushed. Long inflorescence, axillary panicles up to 20 cm long, showy flowers, fragrant, many on slender stalks, white to purple, sepals 5-lobed, 1 cm long, 5-lobed petals, 0.9 cm long, pubescent, stamen tube

Deep Purple blue, 0.5 cm long, 1 cm in diameter. Small fruits, yellow fruit stone, nearly round, about 15 mm, smooth and has a little 'rough, a little' chubby. Seeds are oblongoid shaped, 3.5 mm x 1.6 mm sized, smooth, brown colored and surrounded by pulp. As the leaves decompose, the generic name is derived from the greek word "Melia" (the ash), the specific name derives from the word ‘’azzadirackt’’ (noble tree).

Geographically *Melia azedarach* L. is native in Bangladesh, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Sri-Lanka, Thailand, Vietnam & also exotic in Croatia, Cuba, Djibouti, Dominican Republic, Eritrea, Ethiopia, France, Greece, Guatemala, Honduras, Iran, Iraq, Italy, Jamaica, Kenya, Korea, Republic of, Lesotho, Malta, Mexico, Mozambique, Namibia, Nicaragua, Panama, Paraguay, Philippines, Portugal, Puerto Rico, Saudi Arabia, Singapore, Solomon Islands, Somalia, South Africa, Spain, Swaziland, Syrian Arab Republic, Tanzania, Tonga, Turkey, Uganda, United Kingdom, United States of America, Zanzibar, Afghanistan, Albania, Argentina, Australia, Botswana, Brazil, Brunei, China.

Traditionally *Melia azedarach* L. is used to treat Analgesic, Antibiotic, Antilithic, Diuretic, Deobstruent, Resolvent, Leprosy, Scrofula, Anthelmintics.

2. Material and Methods

2.1 Plant Collection and Identification:

For this present investigation, the fresh leaves of *Melia azedarach* L. was collected from the Naogaon district, Bangladesh in December 2011 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka. (Accession number-35542) and a voucher specimen was also deposited there.

The collected plant parts were separated from undesirable materials or plants or plant parts. They were shade-dried for one week. The plant parts (leaves) were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, China). The powder was stored in a sealed container and kept in a cool, dark and dry place until analysis finished.

2.2 Ethanol Extraction, Filtration with Evaporation:

About 120gm of powered material was taken in a fresh, flat-bottomed beaker and soaked in 550 ml of ethanol. The container with its contents was preserved and kept for a period of 10 days accompanying infrequent shaking and stirring. The whole mixture then undergoes a coarse filtration by a piece of dirt free, white cotton. Then it was filtered through whatman filter paper.

2.3 Phytochemical

Testing of different chemical groups present in extract represents the preliminary Phytochemical studies. The chemical group tests, which are performed as follow. In each test 10 % (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test. Mayer’s reagent, Dragendroff’s Reagent, Fehling’s solution A, Fehling’s solution B, Benedicts Reagent, Molish Reagent, Libermann-Burchard Reagents are used to identify different chemical groups. To test reducing sugar, performed two common tests like Benedict’s test & Fehling’s Test (Standard Test). Ferric Chloride Test and Potassium dichromate test was available for identifying tannins. For testing Steroids, done two familiar tests like Libermann-Burchard test & Sulphuric acid test. Mayer’s test and Dragendroff’s test were applied for tracing alkaloids.

2.4 Experimental Animal

Young Swiss-albino mice aged 4-5 weeks; appropriate average weights (gm) were used for the experiment. They were kept in standard environmental condition for one week in the animal house of Pharmacy Discipline, Khulna University, Bangladesh for adaptation after their acquisition. The mice were provided with typical laboratory food and tap water and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

In animal experiments which was done under the instruction of The Control and monitoring of animals Political and institutional animal (CPCSEA) Committee where Ethics was approved all procedures investment experimental

pain and inflammation Terms of conscious animals.

2.5 Assessment of in-vitro Antioxidant Activity

A Suitably diluted stock solutions were spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1, 1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted. DPPH forms deep pink color when it is dissolved in ethanol. When it is sprayed on the plate, it forms light yellow or yellowish color which indicates the presence of antioxidants.

Basic principle of quantitative analysis- The anti-oxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is very stable free radical which contains an odd electron in its structure and usually utilized for detection of the radical scavenging activity in compound analysis. The aliquots of the diverse concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min, and IC_{50} (Inhibitory conc. 50%) was identified. IC_{50} value refers the concentration of sample required to scavenge 50% of the DPPH free radicals.

The formula used for % inhibition ratio is- % inhibition = (Blank OD-Sample OD/ Blank OD) × 100

2.6 Assessment of in-vivo analgesic activity

The acetic acid induced writhing method[2] is an analgesic behavioral observation assessment method that demonstrates a noxious motivation in mice. The test contains of inject the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (Diclofenac), control and test sample given orally 30 minutes prior to acetic acid introduction. When the sample will give lower number of writhing comparing with control solution at that point we can say it posse's analgesic activity.

Experimental animals were randomly selected and divided into four groups like group-a, group-b, group-c and group-d posses of 5 mice in every group. All groups established a particular treatment seams positive control, control and also two doses of the plant extract. All mice were weighed accurately and the doses of the test samples and control materials were adjusted accordingly.

2.7 Preparation of Sample Suspension: To prepare suspension of the test samples at the doses of 500 mg/kg and 250 mg/kg per body weight, 250 mg and 500 mg of samples were measured correspondingly. The plant extract was triturated in unique manner by the addition of small amount of Polysorbate-80 (Tween-80). After suitable addition of extract and Polysorbate-80 (Tween-80) then added distilled water gradually. Our target was to make the volume of the suspension 5ml up to mark.

For the preparation of Diclofenac Sodium at the dose of 25 mg/kg-body weight, 75 mg of Diclofenac Sodium was taken and volume adjusted to 30 ml.

For preparation of 0.7% acetic acid solution, 0.7 ml glacial acetic acid was taken in a volumetric flask and volume was adjusted to 100 ml with distilled water.

Methodology & Tabulation of Writhing- Feeding needle was used for test samples, control & Diclofenac sodium. For proper absorption, half hour interval was given the administered substances. Then the writhing creating material, acetic acid (0.7%) solution was given intraperitoneally to every mouse. After 5 minutes later, acetic acid absorption was occurred, amount of squirms (writhing) was counted for about 15 minutes.

Each mouse of all groups was observed carefully to count the number of writhing that they had made in 15 minutes. The animal do not always perform full writhing, because sometimes the

animals begin to produce writhing but they do not complete it. This incomplete writhing was considered as half-writhing and two half-writhing were counted as one full writhing. That is why total writhing was halved to convert all writhing to full writhing or real writhing.

3.0 Result

3.1 Photochemical

Results of different group tests are given in the Table no.: 01

Table 1: Phytochemical investigation of *Melia azedarach* L. leaves

Extract	Reducing Sugars	Saponins	Alkaloids	Glycosides	Flavonoids	Tannins	Gums	Steroids
M. azedarach	-	+	+	+	+	+	-	-

"+" = Presence "-" = Absence

3.2 Assessment of in-vitro Antioxidant Activity

The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the existence of antioxidants. The DPPH free radical consist an odd electron which is responsible for the absorbance at 517 nm, and also for visible purple yellow color. When DPPH accepts an electron donated ion by an antioxidant materials, then DPPH is decolorized which shows quantitatively measured from the change in the absorbance.

In the TLC-based qualitative antioxidant assay using DPPH assay, *Melia azedarach* L. leaves showed the free radical scavenging properties indicated by the presence of yellow spot on a purple background on the TLC plate [3].

In the quantitative assay, *Melia azedarach* L. leaves displayed a very free radical scavenging activity in the DPPH assay (IC_{50} = 95.49 approx. µg/ml) which is comparable to that of ascorbic acid (IC_{50} = 15.84 approx. µg/ml), a well-known standard antioxidant**.**

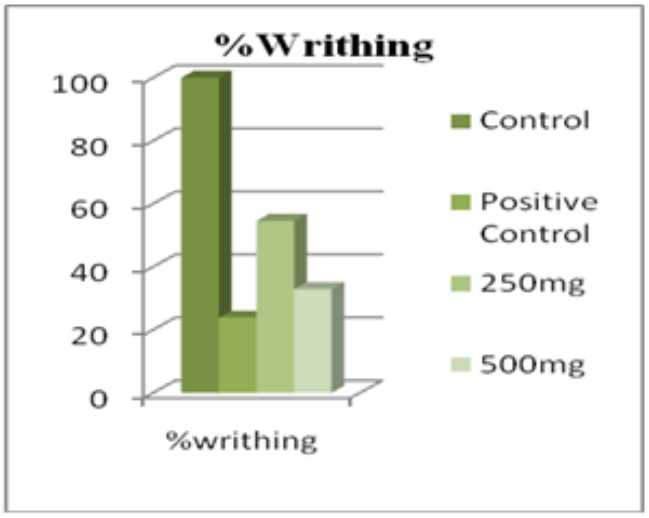
3.3 Assessment of in-vivo Analgesic Activity

Ethanolic extracts of *Melia azedarach* L. (250 mg/kg body weight and 500 mg/kg body weight) exhibited significant inhibition of writhing reflex 45.45% (P<0.001) and 67.05% (P<0.001) respectively while the standard drug Diclofenac Sodium inhibition was found to be 76.14% (P<0.001) at a dose of 25 mg/kg body weight. The test & the result was statistically significant as in all the three cases (positive control, test group- a & b).

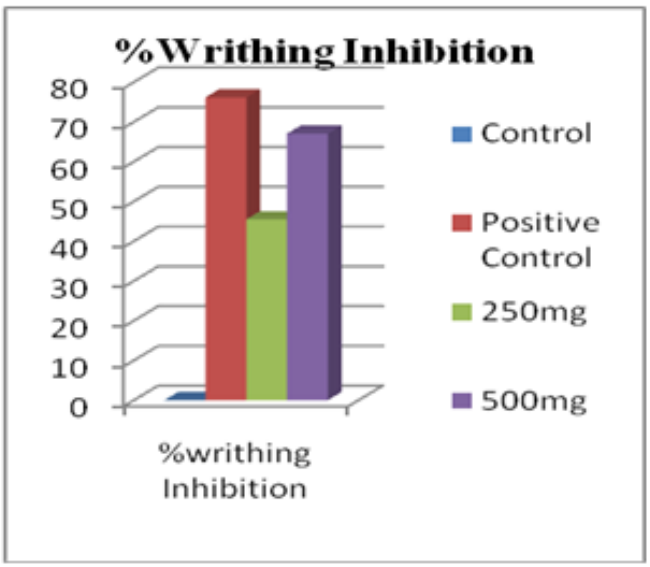
Table 2: Statistical evaluation of *Melia azedarach* L. on acetic acid induced writhing in mice.

Group	**Mice**	**TW**	**MW**	**% Writhing**	**% Inhibition**	**SD**	**SE**	**t- test (P values)**
Control	5	88	17.6	100	-	1.85	0.93	-
PC	5	26	5.2	23.86	76.14	1.23	0.62	11.92 (P<0.001)
250 mg/kg	5	48	9.6	54.55	45.45	1.36	0.68	0.54 (P<0.001)
500 mg/kg	5	29	5.8	32.95	67.05	3.24	1.62	4.53 (P<0.001)

[P.C- Positive Control as Diclofec-Na; T.W- Total writhing; M.W- Mean Writhing; 250 mg/kg- Et. Extract of leaves (250 mg/kg); 500 mg/kg- Et. Extract of leaves (500 mg/kg); SD- Standard Deviation; SE- Stand Error.



[Positive Control as Diclofec-Na; 250mg- Et. Extract of leaves (250 mg/kg); 500mg- Et. Extract of leaves (500 mg/kg)]
Fig 1: Effects of Ethanolic extracts of *Melia azedarach* L. on acetic acid induced writhing in mice.



[Positive Control as Diclofec-Na; 250mg- Et. Extract of leaves (250 mg/kg); 500mg- Et. Extract of leaves (500 mg/kg)]
Fig 2: Percentage writhing inhibition of Acetic Acid induced writhing in mice by the standard drug (Diclofenac Na) & *Melia azedarach* L.

4. Discussion

4.1 Photochemical

The experimental findings from the study showed that the leaves extract of *Melia azedarach* L. possesses organic compounds like- alkaloids, tannins, glycosides, Flavonoids and Saponins which can show extensively pharmacologic & other activities.

4.2 Antioxidant activity

In the TLC-based qualitative antioxidant assay using DPPH assay, *Melia azedarach* L. showed the free radical scavenging properties indicated by the presence of strong yellow spot on a purple background on the TLC plate.

In the quantitative assay, *Melia azedarach* L. leaves displayed free radical scavenging activity in the DPPH assay which is comparable to that of ascorbic acid a well-known standard antioxidant.

4.3 Analgesic activity

Acetic acid is a pain stimulus. Intraperitoneal administration of acetic acid (0.7%) causes localized inflammation. Such pain stimulus

causes the release of free arachidonic acid from tissue phospholipid by the action of phospholipase A_2 and other acyl hydrolases.

There are three major pathways in the synthesis of the eicosanoids from arachidonic acid. All the eicosanoids with ring structures, which is the prostaglandins, thromboxanes and prostacyclines, are synthesized via the cyclooxygenase pathway[4]. The leucotrienes, HETE (hydroxy eicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids) are hydroxylated derivatives of straight-chain fatty acids and are synthesized via the lipooxygenase pathway[5].

The released prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin-E have been reported to be responsible for pain sensation by exciting the Aδ-fibres. Activity in the Aδ-fibres cause a sensation of sharp well localized pain [6].

Diclofenac used as the positive control in this method acts by inhibition of prostaglandin synthesis. Any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.

From the above observation (i.e. Figure 01 & 02) it can be suggested that the ethanolic extract of *Melia azedarach* L**.** *is* an effective analgesic that supports "the plant used as a traditional medicine. However further studies are necessary to elucidate the underlying mechanisms and to isolate and characterize the active constituents responsible for this properties.

5. Conclusion

The present study has demonstrated that Ethanolic extract of *Melia azedarach* L. exhibits valuable analgesic and antioxidant activities. The observations also suggest that the plant contains biologically active constituents that are responsible for the observed actions. However, wide-ranging research is badly needed to get ultimate responsible characteristics.

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