

In-vitro* pharmacological studies and evaluation of phytochemical constituents from the bark of *Melia azedarach

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ABSTRACT

The current investigation deals with the in-vitro pharmacological studies of the bark of Melia azedarach for anti-inflammatory/analgesic and anti-diabetic studies. The preliminary phytochemical studies were also performed for the identification of class of compounds present in the bark. The study reveals the biological potency of the plant and also gave scientific validation for the usage of the plant in Indian System of Medicine.

INTRODUCTION

The human quest for attaining or knowing TRUTH, for attaining knowledge and attaining LONG and HEALTHY LIFE is as old as the human himself. Man started knowing and understanding the things that are helpful to him by gradual and repeated approach to the things present in his surrounding environment. Man made the surroundings adoptable by changing them according to the need of those things to his body[1]. Plants have been utilized as a natural source of medicinal compounds since thousands of year[2.] Medicinal plants are a group of plants used in medicine or veterinary practice for therapeutic and prophylactic purposes[3].

Plants synthesize a bewildering variety of phytochemicals but most are derivatives of few biochemical motifs[4]. Poly phenols (also known as phenolic) are compounds contain phenol rings[5.] Our research group has long been studying the chemistry of highly active Meliaceae Melia Azedarach[6,7,8]. looking for active principle that can be used as safe biopesticides. This tree commonly named pariso ,grows easily in temperature and cold temperature zones of Argentina where it is widespread. It is native to upper Barmah region. It is a original species of south Asia (Iran, India,South of China) Extracts form different plants structure of Melia azedarach have already been studied and shows important anti fungal activity especially the Kernel extract (SKE) [9,10]. Melia is a small genus of 2 species Azedarach & Azadiracta[11]. Melia azedarach belongs to the family Meliaceae is from west Asia. It is widely distributed in Himalayas region between the attitudes of 700- 1000m. A moderate-sized deciduous tree 9-12m in height with a cylindrical bole with dark grey bark having shallow longitudinal furrows[12]. It has been used for various medicinal purposes[13].

Bark used for curing many disease skin conditions, such as eczema, ulcerative wounds, syphilitic ulcer, leprosy, scrofula etc in the form of lotion, ointment or poultice. Systematically it is used as an emetic, cathartic, anthelmintic, antipyretic, expectorant, and diuretic[14,15,16,17.18].

MATERIALS AND METHODS**Scientific classification**

Kingdom : plantae
Division : Magnoliophyta
Class : magnoliopsida
Order : sapindales
Family : Meliaceae
Genus : Melia
Species : M . azedarach
Binomial name : Melia azedarach[19].

Preparation of plant extracts and phytochemical screening

The fresh bark of Melia azedarach subjected for air dried and make up to coarse powder form. Bark powder was extracted successively with methanol using Reflux apparatus. All the extracts were filtered using cotton plug followed by filter paper. The extract were concentrated and dried. The extract were stored in air tight container. The bark extract of Melia azedarach were analysed for the presence of phenols, flavanoids, tannins.

PRELIMINARY PHYTOCHEMICAL SCREENING OF THE MELIA AZEDARACH

The methanolic leaf extract of the bark of Melia azedarach was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

- Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaf extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.]

- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicated the presence of phenols.

Test for flavonoids

- Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

- Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

• Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid

• Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

• Dragendorff's test: A drop of extract was spotted on a small piece of pre-coated TLC plate and the plate was sprayed with modified Dragendorff's reagent. Orange coloration of the spot indicated the presence of alkaloids.
• Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.
• Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides

• Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

• Foam test: 1ml of extract was diluted with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes. A 1cm layer of foam formation indicates the presence of Saponins

Test for Anthraquinones

• Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

• Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper. Oil stains on the paper indicated the presence of fixed oils.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

In-vitro studies of Analgesic Anti-inflammatory activity

The mixture containing 80mM linoleic acid and sufficient amount of potato 5-lipoxygenase enzyme in 50mM phosphate buffer mixture to the substrate and the enzyme activity was monitored as an increase in absorbance at 234 nm using UV kinetic mode on UV-visible spectrophotometer. In the inhibition studies, the activities were measured by incubating various concentrations of test substance with enzyme buffer mixture for 2 minutes before addition of the substrate. The assays were performed in triplicate and mean values were used for the calculation. Percentage inhibition was calculated by comparing slope or increase in absorbance of the test substance with that of controlled enzyme activity. The activity of 5-LOX was compared with the standard positive control Zileutin[20].

In vitro methods employed in Anti-diabetic studies

A total of 500µl of test samples and standard drug (100-100µg/ml) were added to 500ml of 0.20mM phosphate buffer containing amylase (0.5mg/ml) solution and were incubated at 25^oc for 10 min. after these,500ml of a 1% starch solution in 0.02Msodium phosphate buffer was added to each tube. The mixture is then incubated at 25^oC for 10min.the reaction was stopped with 1.0ml of 3,5dinitrosalicylic acid colour reagent. The test tubes were then incubated in boiling water bath for 5min, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle[21,22].

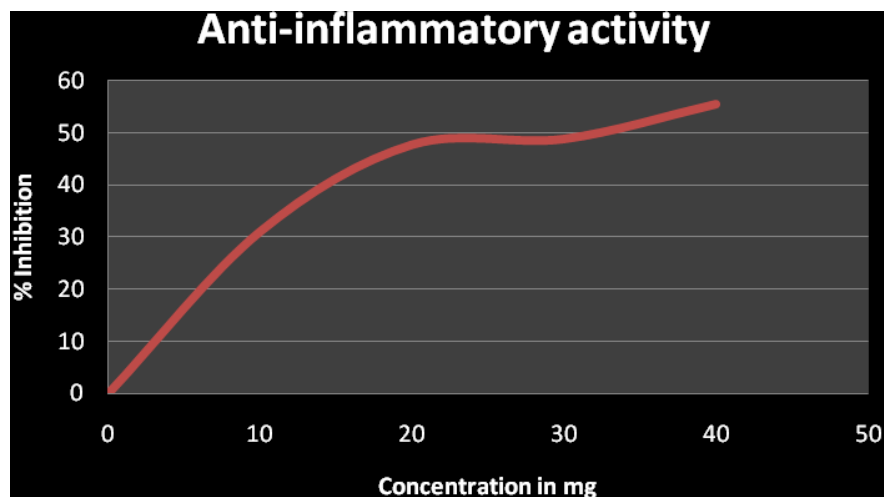
RESULTS AND DISCUSSION

CLASS OF COMPOUND	TESTS PERFORMED	RESULTS
Carbohydrates	Molisch's test	---
	Fehlings test	---
Phenols	Phosphomolybdic acid test	+++
Flavanoids	Shinoda test lead acetate test	+++
Tannins	Braemer's test	+++
Sterols	Salkowski's test	---
Alkaloids	Draggendorf's test	---
Glycosides	Legals test	---
Saponins	Foam test	---
Anthraquinones	Borntragers test	---
Amino acid test	Ninhydrin test	---
Fixed oils and fats		---

Preliminary phytochemical screening of plant methanolic extract.

ANTI-INFLAMMATORY

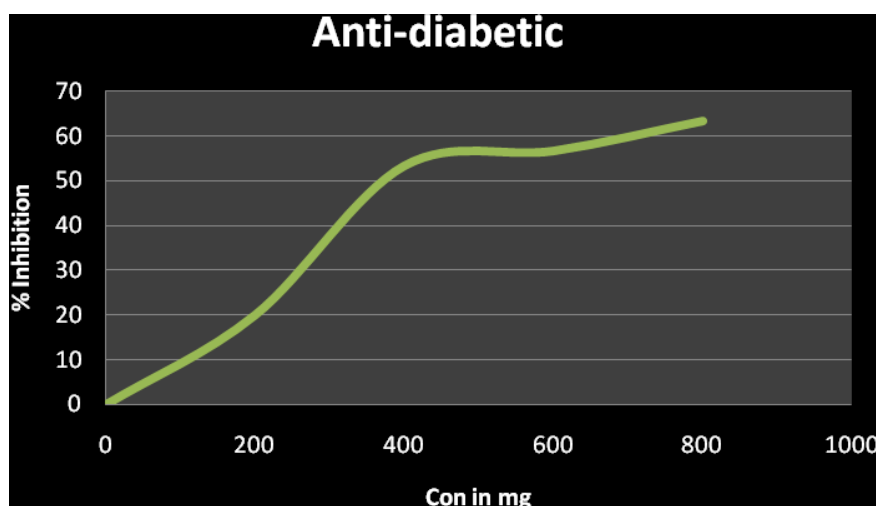
Concentration(mg)	Percentage inhibition
00	00
10	31.11
20	47.77
30	48.88
40	55.55



Graph of anti-inflammatory activity

ANTI-DIABETIC ACTIVITY

Concentration(mg)	Percentage inhibition
0	0
200	20
400	53.33
600	56.66
800	63.33



Graph of anti-diabetic activity

The preliminary test were carried out and from that we come to know that flavanoids,tannins and phenols were in high concentration. The anti-inflammatory study shows an EC 50 of around 30 mg and the in-vitro shows an EC 50 around 250 mg.

CONCLUSION

From the present work we conclude that the bark of the plant *Melia azederach* is a potential drug in terms of biological activity. The plant is being used in ayurvedic system for various ailments. The present work reveals that the chemical compounds present in the bark is highly potential. Further studies can be initiated from the plant like isolation and characterization which will lead to the identification of new biologically potent molecules.

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