IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

STUDIES ON PHYTOCHEMICAL ANALYSIS OF THE LEAVES OF *ANOGEISSUS LATIFOLIA* (ROXB. EX DC.) WALL. EX BEDD). THE MEDICINAL IMPORTANT TREE

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Abstract:

Today India is alarming force in generic world of pharmaceutical market steel in the last twenty years because this is of the country which is a rich storehouse of medicinal plants. All-natural products can be termed bioactive molecules, as every diverse molecule possesses one kind or multiple kinds of biological oblique pharmacological activities. Ethno botanical and traditional uses of natural compounds, especially of plant origin received much attention in recent years as they are well tested for their efficacy and generally believed to be safe for human use. *Anogeissus latifolia* is a deciduous tree widely distributed in tropical and evergreen forests and an important component of agroforestry and food plant of tropical tasar silk worm. In our present investigation phytochemical analysis of *Anogeissus latifolia* young leaves has been evaluated for the presence of bioactive compounds viz alkaloid, Amino acids, Carbohydrates, flavones, phenols, proteins, reducing sugars, saponins, steroids, tannins, triterpenoids and Glycosides. Using various polarity solvents including acetone, benzone, chloroform, and Ether, Methanol, and Distilled water. The study revealed the presence of alkaloid, Amino acids, Carbohydrates, flavones, phenols, proteins, reducing sugars, saponins, steroids, tannins and triterpenoids. The results also suggested that 90% acetone extract of *A. latifolia has* a promising therapeutic potential.

Key words:

A. latifolia , Leaf powder, chemical constituents, Acetone, alkaloids, phytochemical analysis

Introduction:

Plant-derived drugs are used as medicines for treating various diseases for decades. Today we still dependant on “Ayurveda” in about 75% of our medicines. The increasing prevalence of multi-drug resistant strains of the bacteria and the recent appearance of Ebola, Swine Flu, Bird Flu, HIV II and new strains with low susceptibility to antibiotics raises the spectre of the untreatable bacterial infections and add urgency to the search for new infection-fighting strategies. Herbal medicines have recently drawn much attention as an

alternative source of useful drugs for treating or preventing various diseases. *Anogeissus latifolia* is a roadside tree possessing a wide range of biological activities. This medicinal plant is enriched with a variety of phytochemicals, which are widely applicable in curing diverse ailment in human and animals.

The plant species originated from Pacific Island (Singh 2012). It is distributed in temperate and tropical areas of northern hemispheres. In India, it is found in the outer Himalayan region up to 2000ft. It is a large deciduous tree with a height up to 30-35m. The bark is grey, exploiting in somewhat corky scales. Leaves alternate, elliptic-ovate, 5-13 cm long, acuminate, entire, sub-coreacious pinnately veined. Flowers are greenish-yellow, usually male or hermaphrodite, monochlamydeous or rarely polygamous and flowering usually takes place in the month of January to February.

The genus *Anogeissus* belongs to Combretaceae with six species, five native to South Asia, one native to Africa. In India four species A. latifolia, A. acuminate, A. philly rosaefolia and A. pendula have been reported (Brandis 1906). Axlewood (*Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Bedd.) Is a small to medium-sized tree up to 20-36 m tall, with a straight and cylindrical bole up to 80-100 cm in diameter Its wide leaves (that give it the name *latifolia*) are opposite or sub-opposite, simple with grayish-yellow or whitish hairs below. The fruit is a 2-winged pseudo-achene, packed into a dense head with a single seed (Orwa *et al.*, 2009).

Anogeissus latifolia timber gives a heavy hardwood, good charcoal and firewood. It provides a gum that is a good substitute for gum arabic. Its leaves give tannins used for tanning and dyeing. *Anogeissus latifolia* is used as fodder for cattle, buffaloes and other ruminants (Orwa *et al.*, 2009). In certain areas of the subtropical forest of the Himalayan foothills of India, *Anogeissus latifolia* is the most important fodder, fuel and timber tree, and excessive lopping of leaves and cutting of saplings and branches for firewood may cause poor regeneration (Kumar *et al.*, 2006). In these regions, *Anogeissus latifolia* may be a major fodder tree for buffaloes in pastoralist communities (Edgaonkar, 1995)

Distribution

A. latifolia is native to India, Myanmar, Nepal and Sri Lanka, and found throughout tropical Asia. A tree of tropical and subtropical climates, it is found in deciduous or semi-evergreen forests. It is a common element in teak forests but also occurs in the understorey of dipterocarp forests, in bamboo forests. It is also present in vegetation under semi-arid conditions such as savanna woodland and dry rocky hills. It is usually associated with *Albizia lebbeck, Dalbergia* spp., *Grewia tiliaefolia, Albizia amara, Gyrocarpus jacquini* and *Mesua ferrea* (Orwa *et al.*, 2009; Singh, 1982). In India, it grows in most parts of the country except in arid areas and moist areas of North-West India (Singh, 1982). *Anogeissus latifolia* grows up to an altitude of 1200 m, with an average annual temperature of 38-45°C and an average rainfall of 625-2250 mm. It is found on a variety of soil types but prefers deep alluvial soils. It does not tolerate waterlogging (Orwa *et al.*, 2009; Singh, 1982).

Forage management

In India, *A. latifolia* is leafless from February to May, flowers from June to September depending on locality, and mature fruits are present from December to March. Leaf flushing begins in the dry season, peaking before the onset of rains (Orwa *et al.*, 2009).

H. integrifolia, the versatile medicinal plant, is the unique source of various types of compounds having diverse chemical structure. The plant species contains wide range of phytochemicals such as terpenoids, sterols, saponins, tannins, proteins, carbohydrates, and alkaloids in addition *A. latifolia* also contains flavonoids, phenols, cardiac glycosides, coumarins, and quinines.

This research has been conducted for the anti-inflammatory activity with aqueous extract of plant *A. latifolia* in compression to standard drug (Diclofenac sodium). While different extracts of *A. latifolia* were selected to evaluate the cytotoxicity, phytotoxicity, insecticidal and enzyme inhibition activities of this medicinally valuable plant.

Materials and Methods

Fresh leaves of *A. latifolia* were collected during the months of August to September, 2019 (Temperature 28 ±2°C), from Jagityal Karimnagar District, Telangana State. The materials were dried in the shade, powdered and stored in airtight containers.

Preparation of Powder

First the site for leaves collection was decided. The whole leaves were collected from same region of Botanical Garden Department of Botany, SRR Govt. Arts & Science College Karimnagar District. Before picking the whole plant, the soil was moistened. The collection of samples was done in between 30th July 2019 to 10th August 2019. The leaves of *A. latifolia* were separated by scissor then remove the thorns either side of leaves with the help of the blade after at room temperature they were shed dried for 3 days and sun dried for 3days and then milled into coarse powder by a mechanical grinder (Harborne, 1988).

Preparation of Aqueous Extract: - The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No. 42 (125 mm) (Rao *et. al.*, 1995).

Phytochemical Screening:

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

1. **Alkaloid Determination using Harborne (1973) Method**

   5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2. **Test for Amino acids:** To 3 ml of the extract few drops of 0.2 % ninhydrin reagent was added and heated. Formation of violet color indicated the presence of amino acids.

3. Test for Carbohydrates Molisch's test

Molisch's test for Carbohydrates

Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test (Sofowora, 1993).

4. Test for Flavonoids (Flavones)

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973). 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

5. Determination of Total Phenols by Spectrophotometric Method

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. It was measured at 505 nm.

6. Test for Proteins Biuret test:

To 3 ml of the extract few drops of 10 % sodium chloride and 1 % copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.

Millon's test

To 3 ml of the extract few drops of Millon's reagent was added for the formation of red color.

7. Reducing Sugars:

Fehling's test for Combined Reducing Sugars About 0.5 g each portion was hydrolysed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralised with sodium hydroxide solution. To this, few drops of Fehling's solution was added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars (Sofowora, 1993).

8. Saponin Determination

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 550C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath

at about 90^0C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n- butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

9. **Test for Steroids**

2ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

10. **Test for Tannis:** About 0.5 g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

11. **Test for Terpenoids (Salkowski Test)**

5ml of each extract was mixed in 2 ml of chloroform and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish-brown colouration of the interface is formed to show positive results for the presence of terpenoids.

12. Glycosides are natural occurring molecule which carry a sugar group which is bounded by it sanomeric carbon too ther group by a glycosidic bond. Glycosidic bond have the property of bind a sugar molecule to another molecule. Basically, a substachnce which carry a glycosidic bond is known as glycoside. In the structure of glycoside, sugar group is known as glycone and the no sugar group is known as aglycon

(a) Antimony trichloride test: An alcoholic extract of drug evaporate → dry → make extract with chloroform + saturated solution of antimony trichloride in chloroform containing 20% acetic anhydride → appearance of pink colour on heating → presence of steroids and triterpinoids.

(b) Tetranitro methane test: Alcoholic extract of drug + tetranitro methane solution →formation of yellow colour → presence of .sterol and triterpenoid

Results and Discussion

Phytochemical screening of this plant of various extracts showed significant results the results are presented in (Table-1). Reducing sugar present in acetone and ether extract. Steroids and protein were resulted in all extracts except distilled water. Phenol present only in chloroform, methanol and distilled water extract. Alkaloid, Amino acids and carbohydrates present in Acetone and methanol extracts, Triterpenoid present in methanol and distilled water extract. Flavones, proteins and steroids present all extracts expect distilled water. Present in benzene and methanol extract. Tannin was resulted in ether, benzene, and distilled water extract. Saponin is only present in acetone extract.

Fig-I showing the leaves of *Anogeissus latifolia* (roxb. ex dc.) wall. ex bedd) the important medicinally tree a) Younger small tree b) Older Branch

Reddy *et al*. (Reddy, *et.al.,* 1965) reported tannin, (+) leucocyanidin and ellagic acid from the bark, sapwood and heart wood, whereas, Deshapande *et al.* (Deshpande *et.al.,* 1976) isolated 3,3'-di-O-methyle ellagic acid-4'-β-D-Xyloside and 3,4,3'-triO-methylflavellagic acid-4'-β-D-glucoside from stem bark. Steroid, β-sistosterol and a triterpenoid, 3-βhydroxy-28-acetytaraxaren were isolated from the ethyl acetate fractions of levs of *A. latifoli*

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemical characters of the. Deshpande investigated are summarized in (Tables -1) (Fig-I).

Table -1 Photochemical Screening of young Leaves of various extracts of *A. latifolia*

Sl. No	**Chemical components**	**Acetone**	**Benzene**	**Chloroform**	**Ether**	**Methanol**	**Water**
1	Alkaloid	+	-	-	-	+	-
2	Amino acids	+	-	-	-	+	-
3	Carbohydrates	+	-	-	-	+	-
4	Flavones	+	+	+	+	+	-
5	Glycosides	+	+	+	+	+	-
6	Phenolic Acids	+	-	+	-	-	+
7	Protein	+	+	+	+	+	-
8	Reducing sugar	+	-	-	+	-	-
9	Saponins	+	-	-	-	-	-
10	Steroid	+	+	+	+	+	-
11	Tannin	+	+	+	+	+	+
12	Triterpenoid	-	-	-	-	+	+

"+" Present, "-" Absent

The qualitative screening of phytochemical constituents on leaf extract of *A. latifolia* reveals the presence of alkaloid, Amino acids, Carbohydrates, flavones, phenols, proteins, reducing sugars, saponins, steroids, tannins and triterpenoids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bacterial effects (Stray 1998). They exhibit marked physiological activity when administered to animals.

In the present study, the observed alkaloid content in *A. latifolia* could be responsible for their much-acclaimed medicinal values though the exact mode of action is poorly understood. Saponins are a special class of glycosides which have soapy characteristics. It has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponin include formation of forms in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness (Sodipo *et.al.* 2000). These properties bestow high medicinal activities on the leaf extract from *A. latifolia*. Tannins are also known antimicrobial agent. Tannins (commonly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods. Tannins are water soluble plant polyphenols that precipitate proteins. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Sodipo *et.al.* 1991). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et.al.* 1998). Phytotherapeutically tannin containing plants are used to tract nonspecific diarrhoea, inflammations of mouth, throat and slightly injured skins.

Conclusion:

A. latifolia is a deciduous tree belonging to family Combretaceae. It is rich in phytochemicals including alkaloid, Amino acids, Carbohydrates, flavones, phenols, proteins, reducing sugars, saponins, steroids, tannins and triterpenoids. It possesses various ethnomedicinal uses. It possesses various ethnomedicinal uses. Till now, a very less research has been done on this plant. So, a need arises to focus on this plant and its isolated constituents. In this research work, we have tried to portray an updated account of *A. latifolia* with emphasis on its phytomedicines and their clinical studies. As of now, thorough and critical research is being conducted globally to discover novel drugs from unexplored plants, especially from the tropics and sub-tropics. With recurrence of virulent pathogens and their new aggressive mutants, such unique drugs could be the answer to dreadful diseases like malaria, Ebola, flues, AIDS and cancer.

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