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Manoj Kumar Nalla
Department of Botany,
Government Degree College,
Peddapalli, Telangana State and
LBS Mahavidyalaya
Dharmabad, Maharashtra, India

Dr. DR More
Principal, Department of
Botany, LBS Mahavidyalaya,
Dharmabad, Maharashtra, India

Studies on phytochemical analysis of the leaves of *Holoptelea integrifolia* (ROXB.) plant the wonders of a medicinal tree

Manoj Kumar Nalla and Dr. DR More

Abstract

Today India is leading drug maker in world pharmaceutical market occupying noble position within the last twenty years because this is often of the country which may be a rich diversified bank of medicinal plants. All plant derived natural products can be termed biologically active compounds, as every diverse molecule stands one kind or multiple sorts of biological and pharmacological activities. Ethno botanical studies reveals medicinal uses of natural compounds employed by ethnic people, especially of plant derived natural compounds got a excellent extent interest in recent decades as they're well tested for their effectiveness and typically assumed to be safe and sound for human use. In our present investigation phytochemical analysis of *Holoptelea integrifolia* young leaves has been evaluated for the presence of bioactive compounds viz alkaloid, Amino acids, Carbohydrates, flavones, phenols, proteins, reducing sugars, saponins, steroids, tannins and triterpenoids. Using various polarity solvents including acetone, benzene, 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 *H. integrifolia* has a promising therapeutic potential.

Keywords: *H. integrifolia*, leaf powder, chemical constituents, acetone, alkaloids, phytochemical analysis

Introduction

Plant based drugs like Ayurveda drugs used a primitive technique in human civilization, up to middle era, plant drugs was only source for treat various diseases and ailments. From 18th century rapid developments in modern science synthetic drugs slowly occupied plant drugs place. But still the drug discovery system is based on plant chemical analysis of ethno botanical plants and many aboriginal tribes and poor countries still practicing plant based medicine. Recent days very high interests develop on plant based medicine due to synthetic drug harmful effects to human and environment. At the same time very often using of synthetic drugs causes multi drug resistance microbes developed and hardness to life. Recently we seen many type of viral and bacterial strains are evolve and challenge to human life. Overcome to these barriers only solution is discovery of plant medicine. Ethno medicinally used plants phyto chemical analysis give a account of plant drug discovery.

Plant based medicines have a short time ago fraught much concentration a further source of functional drugs for treat or prevent various diseases. *Holoptelea integrifolia* can be a roadside tree possessing an outsized range of biological activities. This medicinally active plant is enriching with a widespread of active phytochemicals, which are extensively pertinent in curative varied ailments in human and animals.

The plant species native place was from Pacific Islands (Singh 2012). It's dispersed in all tropical regions of northern hemispheres. In India, it is found in altogether told mover the India, particularly tropical forest of south India and western India. It's an outsized deciduous tree with a height up to 40-50m. The bark is grey, exploit in rather corky scales. Leaves are alternate, elliptic-ovate, 4-14 cm long, acuminate, entire, pinnate venation. Flowers are mixed colour of green-yellow, flowering commonly takes place within the month of January to February.

Holoptelea integrifolia (Roxb) Plant is one of the wide ranges used medicinally in India *Holoptelea integrifolia* belongs to the Ulmaceae family, generally referred as Indian Elm, natively distributed in tropical regions of Asia, also found in numerous areas of Telangana especially in Adilabad and Karimnagar Districts lies with Maharashtra state border areas.

Corresponding Author:
Manoj Kumar Nalla
Department of Botany,
Government Degree College,
Peddapalli, Telangana State and
LBS Mahavidyalaya
Dharmabad, Maharashtra, India

All parts of this plant accustomed, especially leaves to treat inflammation, bacterial infections, diarrhoea, tumour, diabetes and wound, etc. Bark and leaves of *H. integrifolia* are used as bitter, anthelmintic, astringent, thermogenic, anti-inflammatory, digestive, acrid, carminative and laxative to treat different diseases. Seeds and stem bark applied against ringworms externally. Seeds are conversant in cure ulcers and as body deodorizer (Sharma *et al.* 2001, Durga and Paarakh 2011, Prajapati and Kumar 2003) [14, 4, 10].

The plant has been reported different secondary metabolites, which are active medicinally. Different plant parts like stem bark, heartwood, leaf, seed, pollen, and root are the key sources of assorted medicinally important phytochemicals. Holoptelin-A and B (Rastogi and Melhotra, 1985, Mondal *et al.*, 1993) [12, 8] 2-amino naphthoquinone, friedlin, epifriedlin, β -sitosterol, β -D-glucose, β -amyrin, betulin and betulinic acid are derived from stem bark (Ahmed *et al.* 2013) [1]. 2,3-dihydroxyolean-12-en-28 oic acid, hederagenin are isolated from the centre wood (Misra *et al.*, 1975) [7] while hexacosanol, octacosanol, 1,4-naphthalenidione, α -amyrin (Misra *et al.*, 1997 and a newly invented phytosterol 17-(6-diethylamino) decan-3-yl)-10, 13-dimethyl-12, 13-dihydro-10H-cyclopenta[a] phenanthren-3-ol has been isolated from leaf (Sutar *et al.* 2014). Seeds are the key sources of fatty acids like hexadecanoic acid, myristic, stearic, linoleic, linolenic acid, omega-6 and steroids like stigmasterol, α and β -sitosterol, β -amyrin, friedel-1-en-3-one, lupeol, β -sitosterol-D-glucoside (Biswas and Mallik, 1986) [2]. Histamine, 5-hydroxy tryptamines are isolated from pollen. 24-ethylcholest-22-en-3 α -ol is separated from roots (Jain and, Jain 1988) [6]. Leaves of *H. integrifolia* possess analgesic, anti-inflammation and anti-diabetes properties also used for various skin infections, piles and gastrointestinal diseases (Rizwani *et al.* 2012 and Yoganarasimhan 2000) [13, 20].

H. integrifolia, versatile useable medicinally active plant, is that the irreplaceable source of various classes of compounds having diverse chemical structure. The plant species contains different sort of secondary metabolites like Terpenoids, sterols, saponins, tannins, proteins, carbohydrates, and alkaloids in additionally *H. integrifolia* also posses phenols, quinines coumarins, flavonoids, and cardiac glycosides.

These research aims to return out of varied chemical compounds which are active in medicinally and biologically. *H. integrifolia* in compression to plain drug (Diclofenac sodium). While different extracts of *H. integrifolia* were selected to gauge the cytotoxicity, phytotoxicity, insecticidal and enzyme inhibition activities of this medicinally valuable plant.

Materials and Methods

Fresh leaves of *H. integrifolia* were collected during the months of August to September, 2019 (Temperature 28 \pm 2 $^{\circ}$ C), from forest of Adilabad District, Telangana State. The materials were dried within the shade, powdered and stored in airtight containers.

Preparation of Powder

First the location for leaves collection was decided. The whole leaves were collected from tropical forest region Adilabad district adjoin area of Maharashtra in Telangana State, India. Before collecting sample, the soil was moistened (temperature 28-30). The sample collection was done in between 30th July 2019 to 10th August 2019. The leaves of *H. integrifolia* were separated by scissor then remove the thorns

either side of leaves with blade after at room temperature they were shed dried up to moisture dole out and sun dried for 2 days and then milled into fine coarse powder by grinder (Harborne, 1988).

Preparation of Aqueous Extract: The aqueous extract of every sample was ready by soaked 100 g of dry powder samples in 200 ml of distilled water for 12 h. The extracts were filtered through Whatman paper No. 42 (125 mm) (Rao *et al.*, 1995) [11].

Phytochemical Screening

Detection of phytochemical tests were conducted on the aqueous extract using benchmark procedures to dot out the compounds as designed by Trease and Evans (1989), Harborne (1973) [5], and Sofowara (1993) [17].

1. Alkaloid Determination using Harborne (1973) [5]

Method: Take 5 g of the sample extract into a 250 ml beaker and 200 ml of ethanol with 10% acetic acid was add covered with silver foil and leaved to stand for 4 h. After that filtered and the extract was concentrated with help of water bath to one by fourth of the first volume. Concentrate NH₄OH was poured drop wise to the extract up to the precipitation was complete. Take gape for solution was allowed to stay and the precipitated material was collect and washed by dilute ammonium hydroxide after it filtered. The remained is the alkaloid, which was dehydrated and weighed

2. Test for Amino acids: For estimation of amino acids a small number of drops of 2% ninhydrin reagent mixed to 5 ml of extract solution and heated. Appearance of violet colour indicated presence of amino acids.

3. Test for Carbohydrates Molisch's test: To carry out presence of carbohydrates followed molisch's test in this process 2-5 drops molisch's reagent added to 5ml extract dissolved water in attest tube and shaken well. After that few drops of conc. H₂SO₄ added drop wise through the walls of test tube to formed layer upper side and avoid mixed, then stand for two minutes. Formed red or violet colour layer between the two layers indicated presence of carbohydrates. (Sofowara, 1993).

4. Test for Flavonoids (Flavones): To determine presence of flavonoids in extract sample following methods were used (Sofowara, 1993; Harborne, 1973) [17, 5].

5-6 ml of dilute ammonia solution was added to the 30 ml extract solution and followed added of Conc. H₂SO₄. Yellow layer appeared indicated presence of flavonoids.

2-5 drops 1% aluminium solution added to the extract sample. Yellow coloured appear indicated presence of flavonoids

Determination of Total Phenols by Spectrophotometric Method

The 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.

5. 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 mixed for the formation of red color.

6. 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).

7. Saponin Determination: The method used was that of Obadoni and Ochuko (2001)^[9]. The samples were ground and 20 g of each were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 °C. 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 °C. The concentrate was transferred into a 250 ml separator 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.

8. Test for Steroids: 0.5 g ethanolic extract was added to 2 ml of acetic anhydride with mixing of 2 ml H₂SO₄. The colour turned from violet to blue or green in some samples indicating the presence of steroids.

9. 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 coloration.

10. Test for Terpenoids (Salkowski Test): 5ml of each extract was mixed in 2 ml of chloroform and concentrated H₂SO₄ (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.

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 except 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.

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The

phytochemical characters of the *H. integrifolia* investigated are summarized in (Tables -1).

The qualitative screening of phytochemical constituents on leaf extract of *H. integrifolia* 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)^[18]. They exhibit marked physiological activity when administered to animals.

Table 1: Photochemical screening of young Leaves of various extracts of *H. integrifolia*

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

“+” Present, “-” Absent

In the present study, the observed alkaloid content in *H. integrifolia* 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)^[16]. These properties bestow high medicinal activities on the leaf extract from *H. integrifolia*. 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)^[15]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al.* 1998)^[3]. Phytotherapeutically tannin containing plants are used to tract nonspecific diarrhoea, inflammations of mouth, throat and slightly injured skins.

Conclusion

Holoptelia integrifolia is a deciduous tree belonging to family Ulmaceae. It is rich in phytochemicals including alkaloid, Amino acids, Carbohydrates, flavones, phenols, proteins, reducing sugars, saponins, steroids, tannins and triterpenoids. It possesses various ethno medicinal uses. It possesses various ethno medicinal 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 *H. integrifolia* 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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