Phytochemical screening of *Holoptelea integrifolia* Bark & Leaves Extracts Collected from Agra & Hathras (U.P.)

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**Abstract:**

*Holoptelia integrifolia* Planch is commonly known as Chilib in India. It is distributed in India up to an altitude of 600 m. It has various medicinal uses. *Holoptelia integrifolia* leaves contains 14% proteins which are used as fodder. Bark and leaves are used for treating leprosy, edema, diabetes, intestinal disorders, and other skin diseases. The present study was conducted to evaluate Phytochemical screening of Bark & Leaves extracts of *Holoptelia integrifolia*. The plant extract is obtained by soxhlet extraction method by using Ethanol, Petroleum ether and Chloroform as solvent.

**Keywords:** *Holoptelia integrifolia*, Phytochemical, Leaf extract

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**Introduction**

From ancient times, medicinal plants helps in curing human disease because of bioactive component which are of therapeutic values [Nostro et. al., 2000]. Various disease such as include epilepsy, dysentery, malaria, diarrhea, infantile convolution, fungal and bacterial infections have been cured by the use of these medicinal plants [Sofowora, 1996]. Medicinal plants is considered to be a factory as it contains compounds like alkaloids, glycosides, phenols, saponins, resins, oleoresins, carboxydrates, sesquiterpene, lactones and oils [Singh, 2005] and many compounds have been reported in different species of *Holoptelea integrifolia* such as tannins, terpenoids, proteins, sterols, saponins, carbohydrates and alkaloids [Min et al, 2000, K. Machida et al, 2005]. Use of medicinal plants in India is one of the oldest and most diverse tradition. Approximately 120 or more drugs are used worldwide from 95 plant species [Lewington, 1990]. At present, 12000 or more products isolated so far which are secondary metabolites. These products play an important role in plant defense mechanism against insects, bacteria and fungus [Fransworth, 1976]. *Holoptelea integrifolia* have O.D.A. (Ovipositor Deterent Activity) and P.I.A. (Protease Inhibitor Activity) [Sastri, 1950] and helps in curing bronchitis, obesity and edema [Nasir & Ali, 1985, Baquar, 1995]. Leaves & Bark also have medicinal significance. Juice and mucilage derived from boiling bark is useful against intestinal tumors (Sabin & Bedi, 1983), rheumatism (Bajapi et al., 1995) and is oxytoxic in pregnancy (Tiwari & Padhye, 1993). Stem bark paste is beneficial against ringworm, inflammation of lymph gland, common fever (Singh & Ali, 1994) and scabies. Leucoderma is cured by the paste of leaf (Maheswari & Singh, 1990).

**Material and Method**

1. **Collection of Plant material**

   Different parts of the plant of *Holoptelea integrifolia* (Roxb) Plunch was collected from Agra And Hathras and was confirmed by Dr. J.S. Dhakre and Dr. A. K. Singh (Plant Taxonomist) comparision with Voucher specimen kept in Botanical Department of R.B.S. Collage, Agra and flora of Agra of BSI Dehradun. *Holoptelea integrifolia* was shade dried & finely Powdered to particle size and further used to carry out the extraction and isolation of phyto constituents from selected extracts.

2. **Solvent Used:** Ethanol & Petroleum Ether, Chloroform,
3. Preparation of extracts

By the method of Soxhlet Extraction Crude plant Extract was prepared (Okeke et. al., 2001). About 200 gm of powder was uniformly packed into a Chromatography paper (thimble) and run in Soxhlet extractor. It was exhaustible extracted with 500 ml methanol till 22 cycles will be completed or till the solvent become color less. After that filter the extract with filter paper and in Rotary evaporator solvent evaporate to get the syrupy consistency. Traces of alcohol will be removed by keeping the residue over anhydrous sodium sulphate and finally kept in refrigerator at 4°C to detect antifungal & antibacterial activity

Phytochemical analysis of different crude extract

Extracts were tested for the presence of active principle such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins. Following standard procedures (Debela, 2002) were used –

(i) Alkaloids

Mayer’s test

About 0.5-1 ml of sample was taken in a tube. Few drops of Mayer’s reagent were added. It is shaken and allowed to stand for some time. Appearance of cream color ppt. indicates that alkaloids were present in the sample.

Hager’s test

About 0.5-1 ml of sample was taken in a tube. Few drops (1-2) of Hager’s reagent (saturated solution of picric acid) were added. Appearance of yellow color ppt. after some time mark the presence of alkaloids in the sample.

(ii) Glycosides

Legal test

Sample was treated with small amount of pyridine in a test tube. Few drops of alkaline sodium nitroprusside solution were added. If blood red color appears, then alkaloid was present in the sample.

Sodium nitroprusside test

About 0.5 – 1 ml of sample was taken in a test tube. A pinch of sodium nitroprusside powder and 2-3 drops of sodium hydroxide solution (10 percent) were added. Test tube is shaken and allowed to stand for 2-3 minutes. Appearance of red color indicates presence of glycosides in the samples.

(iii) Tannins and phenolic compounds

Ferric chloride test

Few drops of ferric chloride were added to 0.5 ml of test solution in a test tube. Appearance of blue- green color confirms the presence of tannins and phenols in the samples.

Vanillin Hydrochloride Test

If test solution (0.5 – 1 ml) on treatment with few drops of vanillin hydrochloride reagent gives purplish red color, then tannins and phenols are present in the sample.

(iv) Flavonoids

Shinoda test (Magnesium hydrochloride reduction test)

To the test solution (0.5 0- 1 ml), few reagent of magnesium ribbon were added and concentration hydrochloric acid was added drop-wise. Pink scarlet, crimson and red of occasionally green to blue color appears after few minutes, if flavonoid is present in the sample.

Alkaline reagent test

To the test solution (0.5 – 1 ml), few drops of sodium hydroxide solution (10 percent) were added. Formation of an intense yellow color, which turns colorless on addition of few drops of dilute acid, indicates presence of flavonoids.

(v) Proteins and amino acids

Ninhydrin test
About 0.5 – 1 ml of sample was taken in a test tube it is boiled with 0.2 percent Solution of Ninhydrin (Indane 1, 2, 3, trione hydrate). If violet color appear, then protein is present in the sample.

**Biuret Test**

About 0.5 – 1 ml of sample is taken in a test tube and 2-3 drops or sodium hydroxide solution (10 percent) and 1-2 drops of dilute copper sulphate solution were added. After sometime appearance of violet of pink color confirms the presence of proteins in the samples.

**(VI) Test of steroids and Triterpenoids**

**Salkowski test**

About 0.5 – 1 ml of test solution was treated with chloroform in a test tube. Few drops of concentration sulfuric acid were added, shaken well and than wait for sometime. Appearance of red colour at the lower layer indicates the presence of steroids and formation of yellow lower layer indicates the presence of the triterpenoids.

**Libermann – Buchard test**

Sample (0.5 – 1 ml) was treated with few drops of acetic anhydride in a test tube. Boil and cool, concentration sulphric acid was added from the sides of the test tube, shows a brow ring at the junction of two layers and the upper layer turns green who shows the presence of steroids and formation of deep red color indicates the presence of triterpenoids.

**(VII) Carbohydrates**

**Benedict’s test**

Treated the solution (0.5 – 1 ml) with few drops of Benedict reagent (alkaline solution containing cupric citrate complex) in a test tube. Upon boiling on a water bath, reddish – brown ppt forms, if reducing sugars are present in the sample.

**Fehling’s test**

Equal volume of Fehling’s A (Copper sulfate in distilled water) Fehling’s B (Potassium tartarate and Sodium hydroxide in distiller water) reagents were mixed and few drops of sample were added and boiled. A brick red ppt. of cuprous oxide forms, if reducing sugars are present.

**RESULT AND DISCUSSION**

**Table 1-** Phytochemical studies in extracts of *Holoptelea integrifolia* (Agra)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Plant part</th>
<th>Reducing sugar</th>
<th>Protein</th>
<th>Phenol</th>
<th>Alkaloid</th>
<th>Steroid</th>
<th>Triterpenoid</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Glycosides</th>
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<td>Pt. Ether</td>
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<td>Chloroform</td>
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**Table 2-** Phytochemical studies in extracts of *Holoptelea integrifolia* (Hathras)

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<tr>
<th>Solvent</th>
<th>Plant part</th>
<th>Reducing sugar</th>
<th>Protein</th>
<th>Phenol</th>
<th>Alkaloid</th>
<th>Steroid</th>
<th>Triterpenoid</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Glycosides</th>
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<tbody>
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<td>Pt. Ether</td>
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The results of the phytochemical analysis of the *Holoptelea integrifolia* leaf & Bark extracts in various solvents has shown in Table 1 & 2. Phytochemical investigations on *Holoptelea integrifolia* stem bark of Agra revealed the presence of Proteins, Triterpenoids, Steroids, tannins, Flavonoids, Glycosides, Phenols and reducing sugars. The leaves of *Holoptelea integrifolia* extract showed the presence of steroids, tannins, Flavonoids, Phenols, Alkaloids, Terpenoids, , Glycosides, carbohydrates and protein. Further phytochemical investigations on *Holoptelea integrifolia* stem bark of Hathras revealed the presence of Proteins, Steroids, Terpenoids, Flavonoids, Terpenoids and Tannins. The leaves of *Holoptelea integrifolia* extract showed the presence of Carbohydrate, Proteins, Phenols, Alkaloids, Steroid, Flavonoids, Tannins and Terpenoids.

**Conclusion**

Thus, from the above results the *Holoptelea integrifolia* Bark & leaf extracts showed an plentiful production of Phytochemicals as secondary metabolites and can be used as a potent drug in the pharmaceutical industries. The studies result of the *Holoptelea integrifolia* Bark & leaf extracts gives a basis of its use in medicine to cure bronchitis, obesity and edema, intestinal tumors, rheumatism, oxytocic in pregnancy, ringworm, inflammation of lymph gland, common fever.

**References**