

Preliminary phytochemical screening of certain aphrodisiac plants used in traditional system of medicine

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Abstract

Preliminary phytochemical screening of five aphrodisiac plants has been carried out, study shows the presence of alkaloids, saponin, steroids, flavonoids in ethanolic & aqueous extract of these plant.

Keywords: phytochemical constituents, extraction, medicine, impotency

1. Introduction

Plants are widely used for folk medicinal purpose. Ethanomedicinal Investigation of plants used by various tribes communities all over the world became the focused area of biological researcher's since last few decades. Ancient ethnic communities around the world had learnt to utilize their neighbored herbal wealth for curative as well as offensive purpose (Subramannium and Pushpagadan, 1995) [1]. Number of important modern drugs have been derived from plants which are used by indigenous people (Balick and Cox, 1996) [2]. All plant parts are considered as potential sources of medicinal substances. (Shankar and Ved, 2003) [6].

People have been using medicinal plants for male impotency since time immemorial. Various substances of plant origin have been administered in folk medicine of different culture to energize, vitalize and improve impotency or in fertility. Literature study reveals that significant research works on plants improving fertility or impotency have been carried out (Evans, 1969 [5]; Pallavi *et al.* 2011 [7]; Neychev, 2005 [8]; Thakur *et al.* 2009 [9]; Suresh kumar *et al.* 2000 [10]; Gauthaman *et al.* 2002 [11]; Patel *et al.* 2011 [13]; Sabna *et al.* 2013) [14]. An attempt was made to investigate the preliminary phytochemical screening of following 5 aphrodisiac plants used in traditional system of medicine. Investigated 5 plants are *Curculigo orchoides* Gaertn, *Geodorum densiflorum* (Lam.) Schleg, *Ceropegia bulbosa* Roxb, *Chlorophytum tuberosum* Bak, *Sterculia villosa* Roxb.

2. Methodology

Fresh parts (Leaves, Stem, Seeds and Bark) were collected in different seasons plants were shed dried and plant extracts were prepared using standard method (Kokate *et al.* Harborne, 1998 [3]; Krishnaiah, 2009) [4]. Different qualitative tests were performed for screening the presence of various active plant constituents. These tests are Alkaloids (Mayer's test), Glycosides (Borntragers test), Carbohydrates (Benedicts Test and Barfoed Test), Protein and Amino Acids (Biuret and Ninhydrin test), Flavonoids (Lead acetate test, alkaline reagent test, Shinoda test), Triterpenoids and Steroids (Salkowaski's, Libberman and Burchard's test), Tanin and phenolic

compounds (Ferric Chloride test, Lead acetate test, Iodine solution test, Gelatine test)

3. Extraction

The powdered plant material was extracted with different solvents (Ethanol and Water/Aqua) using Soxhlet apparatus. The crude extract obtained was further dried over water bath. Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compound as prolonged heating may lead to degradation of compounds.

i) Preparation of ethanolic and aqueous extract: Each of the dried powdered and weighed samples was Soxhlet extracted in solvent for 24 hrs and filtered. The whole process was repeated thrice to ensure complete extraction. The extract was filtered while hot and the resulting extract was distilled in vacuum in order to remove the solvent completely and subsequently dried in a desiccator. The extract was weighed and calculated the percentage yield in terms of air dried material.

Detailed phytochemical tests were performed to identify presence of different phytoconstituents.

Test for Carbohydrates

- **Molish Test:** 2 ml of aqueous extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.
- **Fehling's Test:** To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of

reducing sugar.

- **Benedict's Test:** Equal volume of Benedict's reagent and extract were mixed in test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.
- **Barfoed's Test:** 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Test for Protein and Amino acids

- **Biuret's Test:** The extract was treated with 1 ml of 10% sodium hydroxide solution in test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.
- **Million's Test:** 3 ml of extract was mixed with 5 ml of Million's reagent. White precipitate formed which on heating turned to brick red, indicating the presence of proteins.
- **Ninhydrin Test:** 3 ml of the test solution was heated with 3 drops of 5 % Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Test for Glycosides

- **Borntrager's Test:** To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammoniacal layer presence of anthraquinone glycosides.
- **Legal's Test:** 1 ml of test was dissolved in pyridine. 1ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.
- **Keller-Killiani Test:** To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

Test for Alkaloids

To the extract, dilute hydrochloric acid was added, shake it well and filtered. With the filtrate, the following tests were performed.

- **Mayer's Test:** To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.
- **Dragendorff's Test:** To 1-2 ml of filtrate, few drops of Dragendorff's reagent were added in a test tube. Formation of red precipitate indicates the presence of alkaloids.
- **Hager's Test:** To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow

color precipitate indicates the presence of alkaloids.

- **Wagner's Test:** To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins

- **Froth Test:** The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Test for Flavonoids

- **Lead Acetate Test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.
- **Alkaline Reagent Test:** The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.
- **Shinoda Test:** To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicate presence of flavonoids.

Test for Triterpenoids and Steroids

- **Salkowski's Test:** The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.
- **Libermann-Barchard's Test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Test for Tanin and Phenolic compounds

- **Ferric Chloride Test:** Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.
- **Lead Acetate Test:** Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.
- **Dilute Iodine Solution test:** To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicates presence of phenolic compounds.
- **Gelatin Test:** Some quantity of extract dissolved in distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development

of white precipitate indicates presence of phenolic compounds.

Test for Fats and Oils

▪ **Solubility Test:** To 2-3 ml of the alcoholic solution of extract, add few ml of chloroform and solubility was observed.

To 2-3 ml of the alcoholic solution of extract, add few ml of 90% ethanol and solubility was observed.

4. Result & Discussion

Qualitative test were performed and phytoconstituents were observed (Table-1&Table-2). Ethanolic extract showed the presences of Alkaloids, Flavonoids, triterpenoids and steroids almost in all investigated plants. Some phytochemical tests present or absent in ethanolic extract.

Glycosides: Borntrager's test positive as *Geodorum densiflorum*, *Curculigo orchoides*, *Chlorophytum tuberosum*, *Sterculia villosa* (Seed & Bark) Negative as only *ceropegia bulbosa*.

Carbohydrate: Fehling test and Benedicts test positive and Barfoed test show negative for *Geodorum densiflorum*, *Curculigo orchoides*, *Ceropegia bulbosa*, *Chlorophytum tuberosum*, *Sterculia villosa*.

Protein and Amino Acids: Biuret's test and Ninhydrin test show positive in *Curculigo orchoides*, *Chlorophytum tuberosum*, *Sterculia villosa* (Bark) and Negative in *Geodorum densiflorum*, *Ceropegia bulbosa* and seed of *Sterculia villosa*.

Tanin and Phenolic Components: Ferric Chloride test, Lead Acetate test, Dilute Iodine solution test and Gelatin test for positive as *Curculigo orchoides*, *Chlorophytum tuberosum*, *Sterculia villosa*(Bark) and negative as *Geodorum densiflorum*, *Ceropegia bulbosa*, *Sterculia villosa* (Seed).

Following phytochemical tests show positive or negative in

water or aqueous extract.

Glycosides: Borntrager's test shows positive in *Curculigo orchoides* and *Sterculia villosa* (Bark) and negative in *Geodorum densiflorum*, *Ceropegia bulbosa*, *Chlorophytum tuberosum* and Seed of *Sterculia villosa*.

Alkaloids: Mayer's test, Hager's test and Wager's test show positive in *Geodorum densiflorum*, *Curculigo orchoides*, *Ceropegia bulbosa*, *Chlorophytum tuberosum*, Bark of *Sterculia villosa* (Bark) negative as *Sterculia villosa* (Seed).

Carbohydrate: Fehling test and Benedicts test and Barfoed test are positive in *Geodorum densiflorum*, *Ceropegia bulbosa*, *Chlorophytum tuberosum*, *Sterculia villosa* (Bark) and negative as *Curculigo orchoides*, *Sterculia villosa* (Seed).

Protein and Amino Acids: Biuret's Test and Ninhydrin test positive in *Curculigo orchoides*, *ceropegia bulbosa*, *Chlorophytum tuberosum*, *Sterculia villosa* (Bark) and Negative as *Geodorum densiflorum* and seed of *Sterculia villosa*.

Flavonoids: Lead acetate test, Alkaline reagent test, Shinoda Test are positive for *Geodorum densiflorum*, *Curculigo orchoides*, *ceropegia bulbosa*, *Chlorophytum tuberosum*, *Sterculia villosa* (Bark) and negative in *Sterculia villosa* (Seed).

Triterpenoids and Steroids: Salkowaski's Test and libbermann-burchard's test positive as *Geodorum densiflorum*, *Curculigo orchoides*, *ceropegia bulbosa* and *Chlorophytum tuberosum* negative as *Sterculia villosa* (Bark).

Tanin and Phenolic Components: Ferric Chloride test, Lead Acetate test, Dilute Iodine solution test and gelatin test positive as *Curculigo orchoides* and negative in *Geodorum densiflorum*, *Ceropegia bulbosa*, *Chlorophytum tuberosum*, *fSterculia villosa* (Seed & Bark both).

Table 1: Qualitative tests of selected medicinal Plants in Ethanolic Extract

S. No.	Name of the Phytochemical	Name of the test	Geodorum densiflorum (Tuber)	Curculigo orchoides (Root)	Ceropogia bulbosa (Tuber)	Chlorophytum tuberosum (Root)	Sterculia villusa (Seed)	Sterculia villusa (Bark)
1	Glycosides	Borntrager's Test	-ve	+ve	-ve	-ve	-ve	+ve
2	Alkaloides	Mayer's Test	+ve	+ve	+ve	+ve	-ve	+ve
		Hager's Test	+ve	+ve	+ve	+ve	-ve	+ve
		Wager's Test	+ve	+ve	+ve	+ve	-ve	+ve
3	Carbohydrates	Fehling Test	+ve	-ve	+ve	+ve	-ve	+ve
		Benedict's Test	+ve	-ve	+ve	+ve	-ve	+ve
		Barfoed's Test	-ve	-ve	+ve	+ve	-ve	+ve
4	Protiens and Amino Acids	Biuret's Test	-ve	+ve	+ve	+ve	-ve	+ve
		Ninhydrin Test	-ve	+ve	+ve	+ve	-ve	+ve
5	Flavonoids	Lead Acetate Test	+ve	+ve	+ve	+ve	-ve	+ve
		Alkaline Reagent Test	+ve	+ve	+ve	+ve	-ve	+ve
		Shinoda Test	+ve	+ve	+ve	+ve	-ve	+ve
6	Triterpenoids and Steroids	Salkowski's Test	+ve	+ve	+ve	+ve	+ve	-ve
		Libbermann burchard's Test	+ve	+ve	+ve	+ve	+ve	-ve
7	Tanin and Phenolic Compounds	Ferric Chloride Test	-ve	+ve	-ve	-ve	-ve	-ve
		Lead Acetate Test	-ve	+ve	-ve	-ve	-ve	-ve
		Dilute Iodine Solution Test	-ve	+ve	-ve	-ve	-ve	-ve
		Gelatin Test	-ve	+ve	-ve	-ve	-ve	-ve

+ve = Present and -ve = Absent

Table 2: Qualitative tests of selected medicinal Plants in aqueous extract.

S. No.	Name of the Phytochemical	Name of the test	Geodorum densiflorum (Tuber)	Curculigo orchioides (Root)	Ceropogia bulbosa (Tuber)	Chlorophytum tuberosum (Root)	Sterculia villusa (Seed)	Sterculia villusa (Bark)
1	Glycosides	Borntrager's Test	-ve	+ve	-ve	-ve	-ve	+ve
2	Alkaloides	Mayer's Test	+ve	+ve	+ve	+ve	-ve	+ve
		Hager's Test	+ve	+ve	+ve	+ve	-ve	+ve
		Wager's Test	+ve	+ve	+ve	+ve	-ve	+ve
3	Carbohydrates	Fehling Test	+ve	-ve	+ve	+ve	-ve	+ve
		Benedict's Test	+ve	-ve	+ve	+ve	-ve	+ve
		Barfoed's Test	-ve	-ve	+ve	+ve	-ve	+ve
4	Protiens and Amino Acids	Biuret's Test	-ve	+ve	+ve	+ve	-ve	+ve
		Ninhydrin Test	-ve	+ve	+ve	+ve	-ve	+ve
5	Flavonoids	Lead Acetate Test	+ve	+ve	+ve	+ve	-ve	+ve
		Alkaline Reagent Test	+ve	+ve	+ve	+ve	-ve	+ve
		Shinoda Test	+ve	+ve	+ve	+ve	-ve	+ve
6	Triterpenoids and Steroids	Salkowski's Test	+ve	+ve	+ve	+ve	+ve	-ve
		Libbermann burchard's Test	+ve	+ve	+ve	+ve	+ve	-ve
7	Tanin and Phenolic Compounds	Ferric Chloride Test	-ve	+ve	-ve	-ve	-ve	-ve
		Lead Acetate Test	-ve	+ve	-ve	-ve	-ve	-ve
		Dilute Iodine Solution Test	-ve	+ve	-ve	-ve	-ve	-ve
		Gelatin Test	-ve	+ve	-ve	-ve	-ve	-ve

+ve = Present, -ve = Absent

5. Conclusions

Phytochemical screening of 5 medicinal plants reveals that the maximum classes of phytoconstituents present in *Cuculigo orchioides* and *Chlorophytum tuberosum* in ethanolic extract as compared to other plants. The other plants also have equal importance due to presence of major phytoconstituents. Alkaloids and flavonoides are present in both ethanolic as well as aqueous plant extract of *Curculigo orchioides*, *Geodorum densiflorum*, *Cerpegia bulbosa*, *Chlorophytum tuberosum* and seed of *Sterculia villosa*. Bark of *Sterculia villosa* shows absence of alkaloides and flavonoides in aqueous extract. Study will definitely be helpful for scientific validation of investigated plants.

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