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Phytochemical screening of stem extracts of *Argyreia nervosa*

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Abstract
Argyreia nervosa (Convolvulaceae family) is commonly known as elephant creeper. It is well known for its medicinal importance. In the present study extraction of the stem of the plant was performed by three different methods namely maceration, soxhlation and decoction using solvents of different polarity. The extracts were analysed for the presence of various phytochemicals such as alkaloids, flavonoids, anthocyanin, phlobatanin, tannin, proteins and amino acids, carbohydrates, phenol, saponins, anthraquinone, chalcones, glycoside, cardiac glycoside, carotenoids, gums & mucilage, emodin and starch. Maximum percentage yields were obtained from the extract prepared by maceration technique using methanol as solvent. Flavonoid was the dominant biomolecule detected to be present in all the extracts.

Keywords: *Argyreia nervosa*, phytochemicals, maceration, soxhlet, decoction.

Introduction
Plants are considered as a potential source of new drugs from centuries. People have been searching for natural remedies to treat their illnesses since the beginning of time. The discovery of novel, powerful herbal drugs for the treatment of many ailments is the result of the traditional usage of medicinal plants. Approximately seven thousand naturally occurring substances are employed in contemporary medicine; the majority of these have been utilized for centuries by conventional healers, and the annual global market value of medicinal plant products over $100 billion [1]. Plants have been a significant source of newly discovered, pharmacologically active compounds. Many popular drugs have originated from plants, either directly or indirectly. Even in spite of the present focus on using synthetic chemistry to identify and produce medications, plants continue to play a major role in the treatment and prevention of disease. 11% of the 252 medications the WHO classified as basic and necessary were solely derived from plants [2]. Medicinal plants must be appropriately identified and recognized during the development and application of these methods. These methods offer intriguing and cutting-edge viewpoints in the realm of therapeutic plants. There are suggestions made for planning the future function and positioning of medicinal plants in illness prevention [3].
Argyreia nervosa is belonging to Convolvulaceae family is a climbing shrub with woody tomentose stem commonly known as elephant creeper in English and samudra sok in hindi. In India it is found mainly in Deccan, Karnataka and east slopes of the West Ghats at 900 m^3 altitude [4, 5]. It has been used in traditional Ayurvedic medicine for many years to treat a variety of illnesses [6]. In present study attempts have been made to screen the phytochemicals present in stem extracts of *Argyreia nervosa*.

Materials and Methods
Plant sample collection and identification
Plant stem samples were collected from Gadarpur, Uttarakhand, India. Authenticated by Dr. M. N. Reddy, Professor and Head Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat (voucher specimen number: BVBRC206).
After being cleaned with distilled water, the stem was cut into small pieces and allowed to dry in the shade for a period of four weeks. The dried stem were then grinded to fine powder [7].

Extraction

Maceration

10 g of dried powder was used for the extraction with 200 ml of solvent (water, chloroform, ethanol, methanol, hexane, acetone, petroleum ether, hydroethanol). For 72 hours in shaking condition. The process is continued until complete extraction. Remaining dilute solutions of the readily soluble constituents of crude drugs are then dried for 12 hours in hot air oven at 55 °C. Dried powder drug was then collected and dissolved in the respected solvent according to %yield and stored in freeze at 4 °C for further analysis [8].

Decoction

6.25 g of dried powder of stem were used for the extraction with 100 ml of distilled water and boiled until the volume reduce to the 1/4th of the initial volume. Filtrate were collected and dried under hot air oven at 55 °C. Dried powder drug was then collected and dissolved in water according to % yield and stored in freeze at 4 °C for further analysis [9].

Soxhlet extraction

20 gm of dried powder was used for the extraction with 200 ml of respective solvent. This step is repeated until the complete soxhlation. Menstruum were collected and dried under hot air oven. Dried powder drug was then collected and dissolved in respective solvents according to % yield and stored in freeze at 4 °C for further analysis [10, 11]. The percentage yield (w/w %) was calculated from the dried powder that was obtained after evaporation using the given formula [12];

$$\%yield = \frac{Weight\ of\ powder\ obtained\ after\ evaporation}{Weight\ of\ powder\ sample\ taken\ initially} * 100$$

Phytochemical screening

Alkaloids

- **Mayer's method:** 1 ml of obtained extract was added with a drop of Mayer's reagent and observed for the appearance of Creamy white precipitate [13].
- **Hager's method:** 1 ml extract was added to the single drop of Hager's reagent and observed for Creamy yellow precipitate as a positive result [14].

Flavonoids

- **Jone's method:** 1 ml extract was mixed with 1 ml acetone, 1 ml 10% $K_2Cr_2O_7$ and 6 ml 6M H_2SO_4. Positive test was appeared as blue green color [15].
- **Shinoda's method:** 1 ml extract was mixed with 1 ml concentrated HCl. Magnesium strip was added to the reaction mixture. Development of pink scarlet, crimson red or green to blue color indicates positive results [16].

Anthocyanin

- 2 ml extract was mixed with 2 ml 2N HCl and drop of ammonia. Appearance of blue violet color was marked as positive result [15].

Phlobatannin

- 2 ml extract was allow to react with 5 ml 1% HCl until the formation of red precipitate [17].

Tanin

- 2 ml extract was added to the drop of 1% lead acetate and observed for the yellow precipitate [18].

Carotenoids

- **1 ml concentrate HCl:** Phenol (1:1) mixture was added to the 1 ml extract and check for the appearance of green color [15].

Anthraquinone

- 1 ml extract was boiled with conc. $H_2\ SO_4$ until the appearance of fumes. Allow it to cool down at room temperature. Add 3 ml chloroform. Positive test results indicated by the change in color of chloroform layer [18].

Chalcones

- Add 5 ml of extract into 1 ml of ammonia. appearance of red color, indicates the positive results [17].

Carbohydrate

- **Fehling's test:** Take 1 ml extract and add it into the equal volume of Fehling's A and Fehling's B reagents. Boiled the mixture until the formation of brick red precipitate.
- **Barfoed's test:** 1 ml of barfoed reagent was added to the 1 ml extract and mixture was allowed to boil until the formation of red precipitate.
- **Seliwanoff's test:** Add 1 ml extract into 1 ml seliwanhoff's reagent. Then heat the reaction mixture in water bath for few minutes. Observe for the rose red color [19].

Glycoside

- **Borntrager's test:** Mix 2 ml extract with 3 ml chloroform-shake well then separate chloroform, layer and add drop of 10% ammonia. Observed for the pink color [19].
- **10% NaOH test:** Boiled 0.2 ml extract with 1 ml diluted $H_2\ SO_4$ for 15 minutes. Then allow it to cool down before adding 1 ml 10% NaOH and 0.2 ml mixture of Fehling A & B. Brick red precipitate will be formed in the positive test.

Cardic glycoside

- **Kellar-Kiliani test:** Mixed 5 ml extract with 1 ml concentrated $H_2\ SO_4$, 2 ml of glacial acetic acid and 1 drop of ferric chloride. Blue color was appeared in the positive test [15].
- **Baljet's test:** Take 2 ml extract and mix it with drop of baljet's reagent. Yellow-orange color indicates the positive results [20].

Protein and Amino acid

- **Xanthoproteic test:** Add few drops of concentrated nitric acid into 1 ml Extract. Formation of yellow color will be an indication of positive test.
- **Ninhydrin test:** Boil 1ml extract with 1 ml 0.1% acetone solution of ninhydrin solution until the formation of Violet color.
- **Biuret test:** Mix 1 ml extract with 1 ml biuret reagent. Check the test for the purple color formation [18].

Phenol

- **Ferric chloride test:** Take 1 ml extract and mix it with few drops of 5% acidified ferric chloride solution. Observe for the bluish black color [15].

Coumarin

- Mix 2 ml extract with equal volume of 10% NaOH. Then drop a spot-on filter paper and observed under UV light for yellow coloration [15].

Saponin

- **Foam test:** Add 1 ml extract into 20 ml distilled water and shaken well until the stable froth formation [15].
- **Haemolysis test:** Take 1 ml extract and few drops of whole blood. Lysis of red blood cell indicates the presence of saponin.

Gums & mucilage

- Add few drops of extract into 15 ml ethyl alcohol. Then stirred well. Appearance of mucilaginous texture indicates the positive test [15].

Starch

- Mix 1 ml extract with drop of lugol's solution. Blue color indicates the presence of starch in reaction mixture [15].

Emodin

- Add 2 ml extract with 2 ml NH_4 OH and 3 ml benzene. Observe for the red color [15].

Results and Discussion

Percentage yield: The percentage yield, which is calculated as the ratio of the amount of phytoconstituents extracted to the amount of dried sample added to the corresponding solvents, was calculated for the extracts obtained from the three different extraction techniques. This consider the quantity of phytoconstituents lost or deteriorated during the extraction procedure [21]. The data presented in figure 1 align with the findings of Stanojević *et al.*'s (2016) study, which also shows that the maceration extraction method using a methanolic solvent showed the highest yield as compared to the other methods and extraction solvents [22].

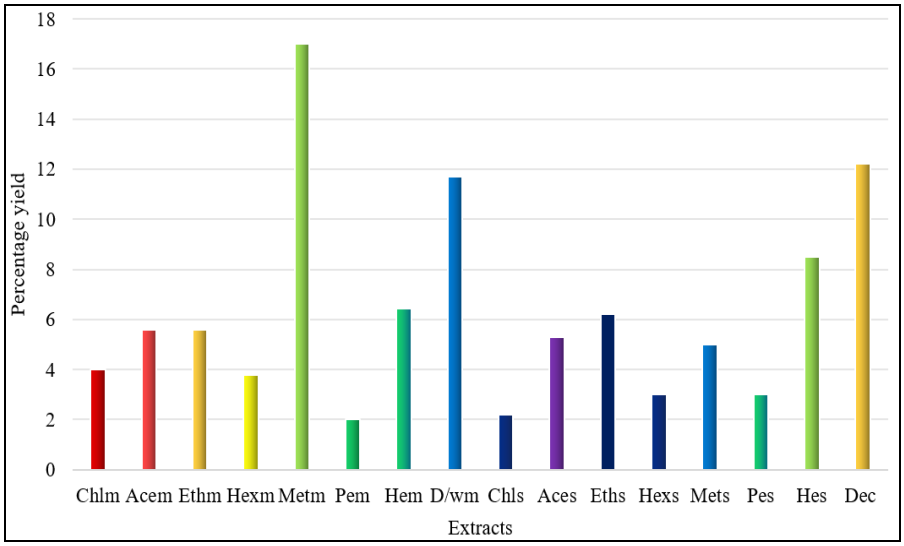

Fig 1: Comparison of percentage yield

Here: Dec: Decoction extract, Stem soxhlet extracts: Hes (hydroethanol), Pes (petroleum ether), Mets (Methanol), Hexs (hexane), Eths (Ethanol), Aces (Acetone), Chls (Chloroform); Stem maceration extracts: D/wm (Aqueous), Hem (hydroethanol), Pem (petroleum ether), Metm (Methanol), Hexm (hexane), Ethm (Ethanol), Acem (Acetone), Chlm (Chloroform).

Phytochemical screening

All the extracts obtained from the three extraction methods using eight polar and non-polar solvents were subjected to phytochemical analysis. The preliminary qualitative screening of all the extracts showed the presence of various groups of phytochemicals such as flavonoids, phlobatannin, tannin, anthraquinone, proteins, phenol, coumarins, saponin and starch (Table 1). There has been a dearth of information about the medicinal role of the stem of this plant but the presence of medicinally active secondary metabolites in the extracts from the stem implied its worth as a potential source of drug [23]. However stem extracts of the plant showed the presence of fewer phytoconstituents than the leaf extracts [4]. These findings also lend credence to the indigenous population's traditional use of this medicinal plant to treat a variety of ailments.

Table 1: Qualitative analysis of phytochemicals of stem of *Argyreia nervosa*

Test	Maceration								Soxhlet extraction							Decoction
	Chl	Ace	Eth	Hex	Met	Pe	He	D/w	Chl	Ace	Eth	Hex	Met	Pe	H	
Alkaloids (Mayer's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids (Hager's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids (Jones's test)	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+	-
Flavonoids (Shinoda test)	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Tanin	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Carotenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Chalcones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrate (Fehling's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrate (Benedict's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Carbohydrate (Seliwanoff's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Glycosides (Borntrager's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides (10% NaOH test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardic glycosides (Kellar-Kiliani test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardic glycosides (Baljet's test)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protein and Amino acid (Xanthoproteic test)	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-
Protein and Amino acid (Nin-hydrin test)	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Protein and Amino acid (Biuret test)	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Phenol	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponin (Foam test)	-	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponin (Haemolysis)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gums & mucilage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Starch	-	-	+	+	-	+	-	-	+	-	-	+	-	++	+	-
Emodin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Here; "+": Presence; "-": Absence; extracts: He (hydroethanol), Pe (petroleum ether), Met (Methanol), Hex (hexane), Eth (Ethanol), Ace (Acetone), Chl (Chloroform), D/w (Aqueous)

Conclusion

The present study reports the presence of medicinally active secondary metabolites like flavonoids, phlobatannin, tanin, anthraquinone, proteins, phenol, coumarin and saponin in the extracts of stem of *A. nervosa*, which can be a source of novel drugs.

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