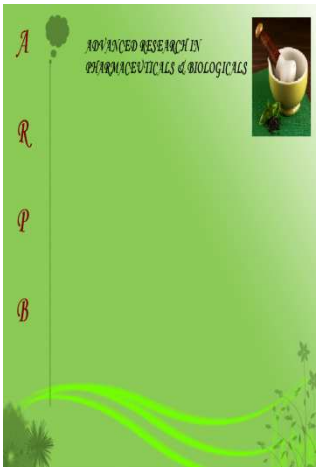
Advance Research in Pharmaceuticals and Biologicals
(A peer reviewed International journal for Pharmaceutical and allied research)

Vol -2 (1)
JAN- MARCH 2012



Received on 06/01/2012
Revised on 16/01/2012
Accepted on23/01/2012

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PHYTOCHEMICAL SCREENING OF *ALECTRA PARASITICA* A. RICH – A RARE MEDICINAL PARASITIC PLANT

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ABSTRACT:

The present paper deals with the phytochemical screening of *Alectra parasitica* A. Rich belongs to family Scrophulariaceae. This study involves the stomatal study of leaf and preliminary phytochemical screening of whole plant powder in various extracts. The extracts showed prominently presence of alkaloids, carbohydrates, sterols, glycosides, saponin, flavonoids, quinone, coumarins and phenolic compounds. The quantitative estimation of three secondary metabolites like alkaloids, flavonoids and saponin has also been carried out.

Keywords: Phytochemical screening, *Alectra parasitica*, Ethnomedicine, Secondary metabolites.

INTRODUCTION

In developing countries, with increasing demand in the field of herbal medicines and cosmetics, it has become necessary and pertinent to probe into the area of systematic knowledge about herbal drugs. There is a need for the application of this knowledge in authentification, detailed study and practical utilization of crude drugs. The use of traditional medicines and medicinal plants in most countries, as a normative basis for the maintenance of good health, has been widely observed[1].

Phytochemical studies have attracted the attention of plant scientist due to development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw material for pharmaceutical industry on the other hand[2]. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. With the development of natural product chemistry, the potential of chemotaxonomy is now becoming increasingly obvious. The application of chemical data to systematic has received serious attention of a large number of biochemists and botanists during the last three decades[3].

Alectra parasitica A. Rich is a erect, herbaceous rare drug plant parasite on the roots of *Vitex negundo* L. which is indigenous to India and had been used in the treatment of leprosy, tuberculosis, paralysis, swellings, fever, expulsion of intestinal worms, constipation for centuries in traditional Ayurvedic medicinal practices, remaining strictly confined to a limited area[4-7]. Only Vaidoos and mendicants practiced with it and it has not been known sufficiently to practitioners of indigenous medicine in other parts of the country. Properties and uses of this drug as known to local people were also recorded. It is felt that this may prove to be a medicinal plant of economic importance.

***Alectra parasitica* A. Rich Parasite on the roots of *Vitex negundo* L.**

***Alectra parasitica* A. Rich in Natural Habitat**

Flowers

So, the present study deals with the laboratory evolutions to assess the phytochemical screening and quantification of secondary metabolites in *Alectra parasitica* A. Rich

MATERIALS AND METHODS

Plant material and its extract: The plant *Alectra parasitica* A. Rich was collected in the month of September from various forest localities of Akola district, Maharashtra. The plant material was taxonomically identified by using standard floras[8-10] and herbarium specimens were deposited in Herbarium of Department of Botany, Shri Shivaji College Akola.

Quantitative Microscopical study: Under this study only the stomatal index value is calculated by using standard procedure[11].

Extraction of plant materials: The collecting plant materials were washed and shade dried. The dried plant material is powdered using mixer grinder, and subjected to Soxhlet extraction with petroleum ether, benzene, chloroform, acetone, ethanol and distilled water respectively for 18h in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening of phytochemicals.

Preliminary phytochemical screening: It involves testing of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug[11-13].

Tests for carbohydrates:

1) Fehling's Test: 1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.

2) Benedict's test: Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was

heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

2) Molisch's test: Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

Tests for proteins:

1) Biuret Test: To the small quantity of extract 1-2 drops of Biuret reagent was added. Formation of violet colour precipitate showed presence of proteins.

2) Million's Test: To the small quantity of extract 1-2 drops of Million's reagent was added. Formation of white colour precipitate showed presence of proteins.

Tests for Anthraquinone glycosides:

Borntrager's Test: To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Tests for Cardiac glycosides:

Keller- Killiani Test: To the 5ml of extract, 1ml of conc. H_2SO_4, 2ml of Glacial acetic acid and 1 drop of $FeCl_3$ solution was added. Appearance of Brown ring shows the presence of cardiac glycosides.

Tests for Coumarins: To the 2ml of extract 10% NaOH was added and shake well for 5 min shows the yellow colour.

Tests for Quinone: To the 2ml of extract conc. H_2SO_4 was added and shake well for 5 min shows the Red colour.

Test for steroids:

1) Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Tests for alkaloids:

1) Hager's Test: To the 2-3 ml of filtrate, 1ml of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

2) Mayer's Test: To the 2-3 ml of filtrate, 1ml of dil. HCl and Mayer's reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids.

3) Dragendroff's Test: To the 2-3 ml of filtrate, 1ml of dil. HCl and Dragendroff's reagent was added and shake well. Formation of orange-brown precipitate showed the presence of alkaloids.

Tests for flavonoids:

1) Shinoda Test: To the extract, added 5 ml of 95% ethanol and few drops of conc.

HCl. To this solution 0.5 g of magnesium turnings were added. Observance of pink coloration indicated the presence of flavonoids.

2) With Lead Acetate: To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Tests for Tannins and Phenolic compounds: 1) $FeCl_3$ Solution Test: On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

2) Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

Test for Saponins:

Foam Test: To 1ml extract 20 ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1cm layer of foam was formed.

Quantitative estimation of secondary metabolite: The presence of secondary metabolites from *Alectra parasitica* A. Rich was quantitatively determined by adopting standard[11-13].

1) Alkaloid estimation: 5 gm of sample was weighed in 250 ml beaker and 200 ml 20% acetic acid in ethanol was added and covered to stand for about 4 hrs. This was filtered and extract was concentrated using water bath to $1/4^{th}$ of original volume. Concentrated Ammonium hydroxide was added drop wise to the extract till its complete precipitation. The whole solution was allowed to settle and precipitate was collected and weighed.

2) Flavonoid estimation: 10 gm of sample was extracted repeatedly in 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann paper no. 42. The filtrate then transferred to a crucible and evaporated to dryness over a water bath and weighed.

3) Estimation of Saponin: 10 gm of plant powder was taken in 200 ml 20% ethanol to make a suspension. This was heated for about 4 hrs over hot water bath (55^oC) continuous stirring. The mixture was filtered and the residue was re-extracted with 200 ml 20% ethanol. The combined extract was reduced to $1/10^{th}$ of the original volume. The concentrate was taken into 250 ml separating funnel, to this added 20 ml diethyl ether and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated for 2-3 times. Then 60 ml n-butanol was added to it. The combined solution was then washed twice with 10 ml 5% aqueous sodium hydroxide. The remnant was heated in a water bath for complete evaporation and dried. This dried content was calculated as Saponin percentage in a sample.

RESULTS AND DISCUSSION

The stomatal study of leaf of *Alectra parasitica* A. Rich shows that there is presence of anomocytic type of stomata found on both lower and upper surfaces. The stomatal index value of lower surface of leaf is higher than that in upper surface. The average highest value of stomatal index observed on lower surface is 11.47% and lowest value observed on upper surface is 4.17% (**Table-1**).

Table 1: Stomatal study of leaf of *Alectra parasitica* A. Rich

Leaf surface	No. of Sites	No. of Stomata	Total no. of Epidermal Cells	% of Stomatal Index	Average % of Stomatal Index
Lower surface	1 2 3 4	8 11 8 9	66 80 75 68	12.12 12.08 10.00 11.68	11.47
Upper surface	1 2 3 4	2 4 3 2	59 68 65 61	3.27 5.55 4.41 3.17	4.17

Table 2: Successive solvent extraction of whole plant powder of *Alectra parasitica* A. Rich

S. No.	Solvent	Colour consistency	Average extractive values (in % w/w on dry wt. basis)
1	Petroleum ether	Yellow oily mass	1.8
2	Benzene	Orange sticky mass	8.2
3	Chloroform	Orange mass	6.0
4	Acetone	Dark orange mass	9.4
5	Ethanol	Brown dry mass	14.0
6	Water	Dark brown dry mass	15.2

While, successive solvent extraction values of *Alectra parasitica* A. Rich in various organic solvents was observed as Petroleum ether 1.8%, Benzene 8.2%, Chloroform 6%, Acetone 9.4%, ethanol 14% and water 15.2% respectively (**Table-2**). The preliminary phytochemical screening of *Alectra parasitica* A. Rich in various extract i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water shows that there is presence of alkaloids, glycosides, saponins, carbohydrates, quinone, coumarins, tannins & phenolics compounds. While, steroids and anthraquinone glycosides are totally absent

in all extracts. Among all phytoconstituents flavonoids, cardiac glycosides, saponine and alkaloids are dominantly present. The majority of phytoconstituents are found in ethanolic and water extracts (**Table-3**). The quantitative estimation of three secondary metabolites (like alkaloids, flavonoids and saponine) also carried out. Out of these three secondary metabolites, flavonoids are present in higher quantity i.e. 25% and saponin percentages are 18%. While, alkaloids are present in lowest quantity i.e. 2.96% (**Table - 4**).

Table 3: Qualitative phytochemical screening of various extract of *Alectra parasitica* A. Rich

S. No.	Constituents	Chemical Tests	Extracts					
			Pet. ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloids	Hager's Test	-	-	+	-	+	+
		Mayer's Test	-	-	-	-	+	+
		Dragendroff's Test	-	-	-	+	+	+
2	Carbohydrates & Glycosides	Fehling's Test	+	+	-	-	+	+
		Benedict's test	-	-	-	+	+	+
		Molisch's Test	+	+	-	+	+	+
3	Steroids	Salkowski Test	-	-	-	-	-	-
4	Saponin	Foam Test	-	+	-	+	+	+
5	Phenolics & Tannin	$FeCl_3$ Sol. Test	-	+	-	-	+	+
		Lead Acetate Test	-	-	-	+	+	+
6	Fixed oil & Fats	Spot Test	-	-	-	-	+	+
7	Proteins	Biurret Test	-	+	-	+	+	+
		Million's Test	-	-	+	+	-	+
8	Anthraquinone glycosides	Borntrager's Test	-	-	-	-	-	-
9	Cardiac glycosides	Keller-Killiani Test	-	+	+	+	+	+
10	Flavonoids	Shinoda Test	-	+	+	+	+	-
		Lead Acetate Test	+	-	+	+	+	+
11	Quinone		-	+	+	-	-	+
12	Coumarins		-	-	+	+	+	+

Table 4: Quantitative estimation of secondary metabolites of *Alectra parasitica* A. Rich

S. No.	Secondary metabolites	Quantity in % (g/100 g dry wt.)
1	Alkaloids	2.96
2	Flavonoids	25.00
3	Saponin	18.00

Note: The results are mean of three determinants

CONCLUSION

The use of plants and various natural products for curing ailments has been a practice for many centuries. Even today the plant products find extensive use in ethanomedicine, traditional system of medicine as well as in the modern physician. Not only developing countries phyto-pharmaceuticals form the main base of national health care programs, but there is global resurgence of interest in medicinal plants. These plants have been recognized by the world health care and importance of scientific investigation of the indigenous herbal medicines has been emphasized. Plants have been one of the important sources of medicine even since the drawn of human civilization. In spite of tremendous development in the field of allopathy during the 21st century, plants still remain one of the major sources of drugs in the modern as well as traditional system of medicine throughout the world.

Various Phytochemical test were carried out and screened out for secondary metabolites from selected plant i.e. *Alectra parasitica* A. Rich is commonly known as 'Nirgunda' belongs to family Scrophulariaceae. It is an important rare medicinal parasitic plant. The present study concluded that, it contains a rich amount of phytoconstituents and showed the presence of alkaloids, glycosides, flavonoids, saponin, quinone, coumarins, tannins and phenolic compounds. So, there is a need to explore its maximum potential in the field of medicinal and pharmaceutical sciences for novel applications.

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