



Phytochemical analysis and Antifungal Activity of *Andrographis Paniculata*

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Abstract

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One of the medicinal plants that seem promising is *Andrographis paniculata* (AP), a shrub found throughout Southeast Asia. It is a well known medicinal plant commonly used in humans as an immune system booster. *Andrographis paniculata* is an herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. The active chemical constituents of *Andrographis paniculata* which have been identified so far include diterpene lactones and flavonoids. The main diterpenoids that have been isolated from *Andrographis paniculata* are 14-deoxyandrographis and 14-deoxy-11, 12-didehydroandrographolide.

The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals. The leaves contain the highest amount of andrographolide (2.39%), the medically most active phytochemical in the plant, while the seeds contain the lowest amount.

Andrographis paniculata has been extensively studied, most of it in the last half of the 20th century and much of it concentrating on "AP's" pharmacological composition, safety, efficacy, and mechanisms of action. A good deal of this research has centered on a screening technique called *signal transduction technology* - probably best explained in a seminal work by Jean Barilla, M.S. The plant pathogenic microorganism *F. oxysporum* (MTCC 7678) was procured from the Microbial Type Culture Collection (MTCC), in Chandigarh. The fungal culture was grown and maintained on a Potato Dextrose Agar medium. The antifungal activity of Andrographolide solution was investigated by using the method of zone of inhibition.

Pure culture of microorganisms that form discrete colonies on solid media, e.g., yeasts, most bacteria, many other micro fungi, and unicellular microalgae, may be most commonly obtained by plating methods such as streak plate method, pour plate method and spread plate method.

INTRODUCTION

Ayurveda is traditional system of medicine of India. It is a qualitative, holistic science of health and longevity, a philosophy and system of healing the whole body, mind of individual (Mukharji *et al.*, 2005). Many herbal secondary metabolites, chemical compounds and formulations have been studied for their biological actions related to prevent human disease (Krushna *et al.*, 2009).

Andrographis paniculata, the Kalmegh of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in north-

eastern India as 'Maha-tita', literally 'king of bitters' and known by various vernacular names. It is also known as 'Bhui-neem', since the plant, though much smaller in size, shows similar appearance and has bitter taste as that of Neem (*Azadirachta indica*). The genus *Andrographis* consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *A. paniculata* is the most popular.

Description

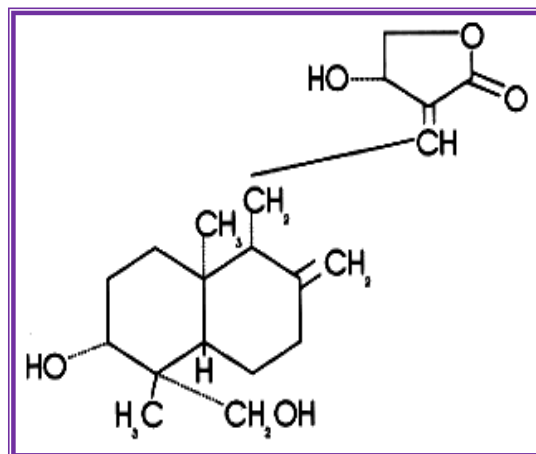
It grows erect to a height of 30-110 cm in moist shady places with glabrous leaves and

white flowers with rose-purple spots on the petals. Stem dark green, 0.3 - 1.0 m in height, 2 - 6 mm in diameter, quadrangular with longitudinal furrows and wings on the angles of the younger parts, slightly enlarged at the nodes; leaves glabrous, up to 8.0 cm long and 2.5 cm broad, lanceolate, pinnate; flowers small, in lax spreading axillary and terminal racemes or panicles; capsules linear-oblong, acute at both ends, 1.9 cm x 0.3 cm; seeds numerous, sub quadrate, yellowish brown.

Distribution

A. paniculata is distributed in tropical Asian countries often in isolated patches. It can be found in a variety of habitats i.e. plains, hill slopes, waste lands, farms, dry or wet lands, sea shore and even road sides. Native populations of *A. paniculata* are spread throughout south India and Sri Lanka which perhaps represent the centre of origin and diversity of the species. The herb is also available in northern stations of India, Java, Malaysia, Indonesia, West Indies and elsewhere in Americas where it is probably introduced in India, including the plains and hilly areas up to 500 m, which accounts for its wide use. Since time immemorial, village and ethnic communities in India have been

using this herb for treating a variety of ailments.



Andrographolide

Morphology and chemistry

Andrographis paniculata is an annual - branched, erect - running 1/2 to 1 meter in height. The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals.

The leaves contain the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest. The primary medicinal component of *Andrographis* is andrographolide. It has a very bitter taste, is a colorless crystalline in appearance, and is called a "diterpene lactone" - a chemical name that describes its ringlike structure (see diagram at left). Besides the related bitters cited above,

other active components include 14-deoxy-11, 12-didehydroandrographolide (andrographolide D), homoandrographolide, andrographan, andrographon, andrographosterin, andstigmasterol. Extraction is usually performed using ethanol, and liquid extracts or tinctures are the most common form of dispensing the product. When consumed, andrographolides appear to accumulate in organs throughout the viscera.

Known Mechanism of action

Andrographis paniculata has been extensively studied, most of it in the last half of the 20th century, and much of it concentrating on "AP's" pharmacological composition, safety, efficacy, and mechanisms of action. Many of the steps involved in signal transduction are well understood, although research can be done to fine-tune an understanding of these pathways. Investigating what can go wrong at such a basic level (inside the cell) allows researchers to detect diseases at a much earlier stage -- before there are obvious symptoms and when there is still a good chance to correct the problem.

"Scientists at many U.S. companies are using signal transduction technology to determine the effects of natural and synthetic components on the signal transduction pathways in the cell, in particular those involved in cell division... Several applications of signal transduction technology in the development of compounds with therapeutic potential have been reviewed in an excellent editorial published in *Genetic Engineering News* in January 1996.

"Several studies have looked at the disposition of andrographolide in various organs of the body. Biodistribution experiments have been done in experimental animals. Following injection of radioactively labeled andrographolide, this compound appears to be widely distributed in the body. High concentrations are noted in the central nervous system (brain and spinal cord) and other organs with high blood flow, including the colon, spleen, heart, lungs, and kidneys. Andrographolide appears to have a relatively short half-life of approximately two hours. The term "half-life" refers to the time when the concentration of the compound in the body is half of what it originally was when it

entered the body. This is what is left after the compound has been metabolized (broken down), changed into other forms (called metabolites), and excreted by one of several routes (urine, feces, exhaled air, sweat, or other body excretions). Compounds with short half-lives need to be given often since they do not stay in the body for long. Andrographolides are excreted fairly rapidly from the body via the urine and gastrointestinal tract. In some studies, 80 percent of the administered dose of andrographolide is removed from the body within eight hours, with excretion rates of more than 90 percent of the compound within forty-eight hours.

Introduction of Fungus

Fusarium oxysporum also referred to as panama disease or agent green, is a plant pathogenic fungus that causes 'Fusarium wilt' in more than a hundred species of plants such as tomato, potato, sugarcane, cowpea, *Musa spp.*, pea, ginger, etc. It colonizes the xylem of the host plant, and as a result, blockage and breakdown of the xylem leads to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant. Management of *F. oxysporum* is required, as this pathogen and

its many special forms affect a wide variety of hosts of economic value.

The development of resistance to common fungicides and increasing restrictions on the use of toxic material in the environment has given an impetus to the search for novel plant protectants that interfere with the fungal pathogenicity factors. Use of natural products for the control of fungal diseases in plants is considered as an alternative to synthetic fungicides, due to their lower negative impacts on the environment. Besides being harmless and nonphytotoxic, it has been proved that plant extracts exhibit effects on germination and on the viability of fungal spores as well. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and nonphytotoxic, unlike chemical fungicides. namely, *Lantana camara* L. (Verbenaceae), *Tridax procumbens* L. (Asteraceae) and *Capparis decidua* Forsk (Edgew) (Capparaceae) were selected in the present study, for evaluation of their antifungal activities. The selected plants were well adapted to harsh (xerophytic) climatic conditions and were well known, among local natives, for their medicinal properties.

MATERIALS & METHODS

Phytochemical analysis and qualitative determination of chemical compounds of *Andrographis paniculata*.

Isolation of Andrographolide

Antifungal activity of Andrographolide

Objective

The objective of this experiment was to analyse the quality of the sample of AP leaves to assure that they contained sufficient active compounds and isolate Andrographolide.

Materials and methods

The phytochemical compounds of this powder were analyzed by colour test (preliminary test) and thin layer chromatography (confirmatory test).

Extraction of AP Leaves by successive extraction method:

Soxhlet continuous extraction apparatus: In this apparatus extraction is boiling solvent followed by percolation finally evaporation yields the extract and recovered solvent ready for the next sample.

Extraction of *Andrographis paniculata* using solvent-

Hexane: Take 77.505 gm dry *Andrographis paniculata* and extracted to 8.30hrs. The extracted yield is 0.47gm.

DCM: Take 77.00 gm dry *andrographis paniculata* after hexane extraction extracted by DCM (dichloro methyl) for 5hrs, and extracted yield is 1.211gm.

MeOH: In last extraction done by Methanol. In this from 75.01 gm of *andrographis paniculata* extracted for 4.30 hr extract yield is 1.318 gm.

Preliminary test

Test for Alkaloids: Weigh about 0.2 gm of plant extract in separate test tube and warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragencloff's reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

Keller-Killani Test: Weigh about 0.5 gm of plant extract in a separate test tube with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1 ml of concentrated tetra oxo

sulphate (VI) acid. And observe for brown ring formation at the interface (Finar, 1983).

Test for Terpenoids: Weigh about 0.5 g plant extract in separate test tubes with 2 ml of chloroform. And add concentrated Sulphuric acid carefully to form a layer. And observe for presence of reddish brown color interface to show positive results for the presence of terpenoids.

Test for reducing sugars: Take a test tube and add 2 ml of crude plant extract and add 5 ml of Distill water and filter. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 minutes. Observe for orange red precipitate which indicates the presence of reducing sugars.

Test for Saponins: Weigh about 0.2 gm of plant extract in the test tube and add 5 ml of distilled water and then heat to boil. Observe for the occurrence of frothing (appearance of creamy mass of small bubbles) which then indicates the presence of Saponin.

Test for Tannin: To small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate.

And observe for dark green solutions that indicate the presence of tannin.

Test for Carbonyl: Take 2 ml of plant extract in separate test tubes and add few drops 2,4, di nitro phenyl hydrazine solution and shake. And observe for the presence of yellow crystals immediately for the presence of an aldehyde.

Test for Flavonoids: Weigh about 0.2 gm plant extract in separate test tubes and dissolved diluted Sodium hydroxide and add diluted Hydrochloride. And observe for yellow solutions that turn colorless. This indicates the presence of flavonoids

Test for Phlobatanin: Weigh about 0.5 gm of plant extract in a test tube and dissolve with distilled water and filter. The filtrate was boiled with 2% Hydrochloric acid solution. Observe for a red precipitate that shows the presence of Phlobatanin

Test for Steroids: To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of Sulphuric acid .Observe for the color change from violet to blue or green in samples indicating the presence of steroids

Confirmatory test

Thin layer chromatography analysis was done after the colour test to confirm the AP identity. 1 g of powdered AP leaves was boiled with 20 ml of ethanol in a water-bath for 5 minutes, and then 300 mg of decolorizing charcoal were added, stirred and filtered. The filtrate was evaporated under reduced pressure until dryness and the residue dissolved in 1 ml of warm ethanol (80%). As standard 2 mg of andrographolide, 2 mg of neoandrographolide and 4 mg of dehydroandrographolide were dissolved each in 1 ml of ethanol by using adsorbent silica gel GF254 and mobile phase chloroform (absolute ethanol 85:15). 5 micro liters was used for 40 each spot. Migration path was 15 cm (ascending). After quenching by UV radiation ($\lambda=254$ nm) detection was made by spraying with 2% w/v 3, 5-dinitrobenzoic acid excess of 5.7% w/v potassium hydroxide.

Work on Hexane part

The hexane fraction was chromatographed over silica gel (100gm) eluting with hexane, and eluants increasing polarities using varying proportion of EtOAc and MeOH to provide 1-2.

The Column chromatography was performed on silica gel and spots were visualized by exposure to I_2 vapours and or 10% sulphuric acid sprays, followed by heating at 110°C. Details of fractions of hexane extract are given in Table 2.

Fraction A was rechromatographed and provided a viscous mass compound AP-1, $[\alpha]_D^{+0.98}$ R_f 0.56 (CHCl₃:hexane 10:90). By comparison of its physical and spectral data with their literature, it was confirmed as trans-phytol.

Fraction F was further chromatographed 14-deoxyandrographolide and provided a solid compound AP-2, mp. 171°C $[\alpha]_D$, -28.5°. R_f 0.50 (MeOH:EtOAc 3:97), crystallized from Methanol. By comparison of its physical and spectral data with their literature, it was confirmed as 14-deoxyandrographolide.

Work on EtOAc part

The EtOAc fraction was chromatographed over silica gel eluting with hexane, and eluants increasing polarities using varying proportion of EtOAc and MeOH to provide compound 3.

The column chromatography was performed on silica gel(60-120 mesh) and the spots were visualized by exposure to I₂ vapour and or 10% sulphuric acid spray, followed by heating at 110⁰C. Details of column chromatography of ethyl acetate are following.

Details of pooled fractions

Fraction C was chromatographed and yielded a compound AP-3, mp. 172-221⁰C[α] D, -92⁰C, Rf

0.46(MeOH:CHCl₃, 5:95), crystallized from methanol. By comparison of its physical and spectral data with their literature data, it was confirmed as andrographolide.

Method to identify the active constituents (diterpene lactones) of AP powder.

Antifungal activity

Material and methods

The plant pathogenic microorganism *F. oxysporum* (MTCC 7678) was procured from the Microbial Type Culture Collection (MTCC), in Chandigarh. The fungal culture was grown and maintained on a Potato Dextrose Agar medium. The antifungal activity of Andrographolide solution was

investigated by using the method of zone of inhibition.

MEDIA PREPARATION

Steps involved in the preparation of PDA medium are

- 20 ml of sterilized distilled water is taken into a clean conical flask.
- 40 gm of potato infusion is added.
- 4 gm of dextrose is added.
- Into this 3 gm of agar is added.
- The solution is well mixed and the solution is made up to a volume of 200 ml by adding distilled water.
- The solution is heated on a hot plate for the proper dissolution.
- The medium is sterilized by autoclaving at 15 psi pressure, 121⁰C temperature for 15 minutes.

Common Methods of isolation of pure culture

Pure culture of microorganisms that form discrete colonies on solid media, e.g., yeasts, most bacteria, many other microfungi, and unicellular microalgae, may be most commonly obtained by plating methods such as streak plate method, pour plate method and spread plate method.

Inoculation Procedure

The steps involved in the inoculation procedure are;

1. The tube containing inoculums and the tube containing agar slant are held in the left hand and the inoculation loop/needle in the right hand. Tubes should almost be parallel to the ground to avoid contamination.
2. Both the tubes are opened by removing the cotton-plug with fingers of the right hand and the open mouth of the tubes is sterilized by passing through the flame twice.
3. Immediately after unplugging and sterilizing the mouth of the tubes, the loop/needle is also flame-sterilized and is inserted within the agar surface of the inoculums containing tube to quench the heat, and a small bit of inoculums is taken on the loop/needle tip.
4. The inoculums containing loop/needle is taken out and brought in within the agar slant containing tube where the inoculums are just rubbed on the surface of the agar slant.

5. All the steps starting from plug removal from the mouth of the tubes to the rubbing of the inoculums on the surface of the agar slant should be taken quickly to avoid contamination.

6. When inoculation is complete, the open mouths of tubes and the cotton plugs are sterilized by flame and the cotton plugs are replaced.

7. The inoculated tube is incubated under suitable temperature to favour rapid growth of microorganisms.

Antagonistic activity of *Andrographolide* against *A. nigar*

After the inoculation procedure of Fungus prepared solution/dilution of andrographolide (1.5mg in 100 μ l) is taken and in Petri plate dipped 4 disc of filter paper. After them marked as one control, On 2nd applied 10 μ l, on 3rd applied 20 μ l and on 4th filter disc applied 40 μ l and incubated for 48hrs.

After 48 hrs saw zone of inhibition and measured them.

Antagonistic activity of *Andrographolide* against *fusarium*

Same procedure follows against *fusarium*.

RESULTS AND DISCUSSION

Results of confirmatory test

The results of the thin layer chromatography are given in Table 6.

Results of chemical content of AP

The following results were obtained: foreign matter 0%, water 8.58%, acid-insoluble ash 1.39%, 85% ethanol extractives 18.10% and active constituents (total diterpene lactones calculated in andrographolide) 7.30%.

Discussion

The primary tests which only show the presence of compounds which reacts chemically like the active compounds of AP. To prove that the sample contain AP further tests in the laboratory are necessary. The thin layer chromatographic test showed that the AP plant sample had the same Rf values (line spots and colour) as the diterpene lactones standards which were used for comparison in this test. So it can be stated that the plant sample contains the three active compounds of AP plants: dehydroandrographolide, andrographolide and neoandrographolide. The TLC method is used to confirm the result from the colour

test by the same offices which do the colour test.

Result of Isolation of Andrographolide

The EtOAc fraction was chromatographed over silica gel eluting with hexane, and eluants increasing polarities using varying proportion of EtOAc and Methanol to provide compound AP-1 to AP-3. The column chromatography was performed on silica gel (60-120mesh) and spots were visualized by exposure to I₂ vapours and or 10% sulphuric acid sprays, followed by heating at 110C.

Compound Ap-1

Fraction from 5% EtOAc: Hexane solvent system, compound Ap-1 was obtained. $[\alpha]_D^{+0.98}$. It was homogenous on TLC plate in different solvent systems, Rf 0.43 (ethyl acetate:hexane, 10:90). IR spectrum of compound AP-1 showed the presence of hydroxyl group at 3300(O-H), 1670(C=C) 790cm⁻¹. ¹H-NMR(CDCl₃) of compound AP-1 showed (3H, s, >C=CH₃) at δ 1.65 and (2H, d, J=7Hz, =C-CH₂-O-) at δ 4.12. ¹³C-NMR of the compound AP-1 showed the signals at 123.56 and 138.12 for C-2 and C-3 respectively.

A molecular ion peak of the compound AP-1 was observed in the mass spectrum at m/z 296 which corresponded to the molecular formula $C_{20}H_{40}O$. The other ion fragmentations at m/z 278, 262, 248, 235, 222, 209, 237, 121, 98, 85, 81, 71, 57, 43. On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as trans-phytol.

Compound Ap-2(14-deoxy Andrographolide):

Fraction from 1% MeOH: EtOAc solvent system, a colourless solid was obtained, crystallized from MeOH, mp. 172-173 $^{\circ}C$, $[\alpha]_D -28.5^{\circ}C$. It was homogenous on TLC plate in different solvent systems, R_f 0.50 (methanol: ethyl acetate, 3:97). IR spectrum of compound AP-1 showed the presence of hydroxyl group at 3630, α, β – unsaturated γ -lactone portion at 1755, double bond at 1640 cm^{-1} . 1H -NMR($CDCl_3$) spectrum of compound AP-2 showed the methyl group at δ 0.62 and δ 1.22 and exomethylene group at δ 4.55 δ 4.84 and δ 4.84.

A molecular ion peak the compound AP-2 was observed in the mass spectrum at m/z 334 which corresponded to the molecular

formula $C_{24}H_{34}O_4$. On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as 14-deoxy andrographolide.

Compound AP-3 (Andrographolide):

Fraction from 5% MeOH:EtOAc solvent system, a colourless solid was obtained, crystallized from methanol, mp. 172-221 $^{\circ}C$, $[\alpha]_D -92^{\circ}C$. It was homogeneous on TLC plate in different solvent system, R_f 0.46 (methanol: ethyl acetate, 5:95). IR spectrum of compound showed the presence of hydroxyl gp at 3340-3200, α, β – unsaturated γ -lactone portion at 1725, double bond at 1667 cm^{-1} . 1H -NMR ($CDCl_3$) spectrum of compound AP-3 showed the methyl gp at 0.86 and 1.07 and the exomethylene gp at δ 4.58 and δ 4.77.

A molecular ion peak of the compound AP-3 was observed in the mass spectrum at m/z 350 which corresponded to the molecular formula $C_{20}H_{30}O_5$. On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as andrographolide.

Result of Antifungal activity of Andrographolide

From the table 9. It is clear that Andrographolide is active against *Aspergillus* and *Fusarium*. We can also seen that as we increasing concentration zone of inhibition increasing. Now we can conclude Andrographolide show antagonistic property against fungus and it show much antagonistic property against *Fusarium* comparison *Aspergillus*

CONCLUSION

From Table 6 confirmatory test it is clear that leaf extract of *Andrographis paniculata* showed spots of different Rf values b/n 28-32, 52-56,69-71.It is well known that Andrographolide gives Rf value b/n 52-56.

So from confirmatory test it is clear that extract of *Andrographis paniculat* contain Andrographolide.

From table 8 it is concluded that compound AP-3 obtained from column chromatography is Androgeapholide.

It is well clear from Mass and NMR that: Fraction from 5% MeOH:EtOAc solvent system, a colourless solid was obtained, crystallized from methanol ,mp.172-221⁰C, ,[α] D ⁰C -92 ⁰C.It was homogeneous on TLC plate in different solvent system, Rf

0.46(methanol: ethyl acetate,5:95).IR spectrum of compound showed the presence of hydroxyl gp at 3340-3200, α , β – unsaturated γ -lactone portion at 1725, double bond at 1667 cm^{-1} . ¹H-NMR (CDCl₃) spectrum of compound AP-3 showed the methyl gp at 0.86 and 1.07 and the exomethylene gp at δ 4.58 and δ 4.77.

A molecular ion peak of the compound AP-3 was observed in the mass spectrum at m/z 350 which corresponded to the molecular formula C₂₀H₃₀O₅.On the comparison of its physical and spectral data with those reported in the literature, it is confirmed as andrographolide. So it is fully proved that compound AP-3 is Andrographolide. For antagonistic activity it clears that: From the table6.4 it is clear that Andrographolide is active against *Aspergillus* and *Fusarium*. We can also seen that as we increasing concentration zone of inhibition increasing. Andrographolde is most active against *fusarium*. Now we can conclude Andrographolide show antagonistic property against fungus and it show much antagonistic property against *Fusarium* comparison *Aspergillus*.

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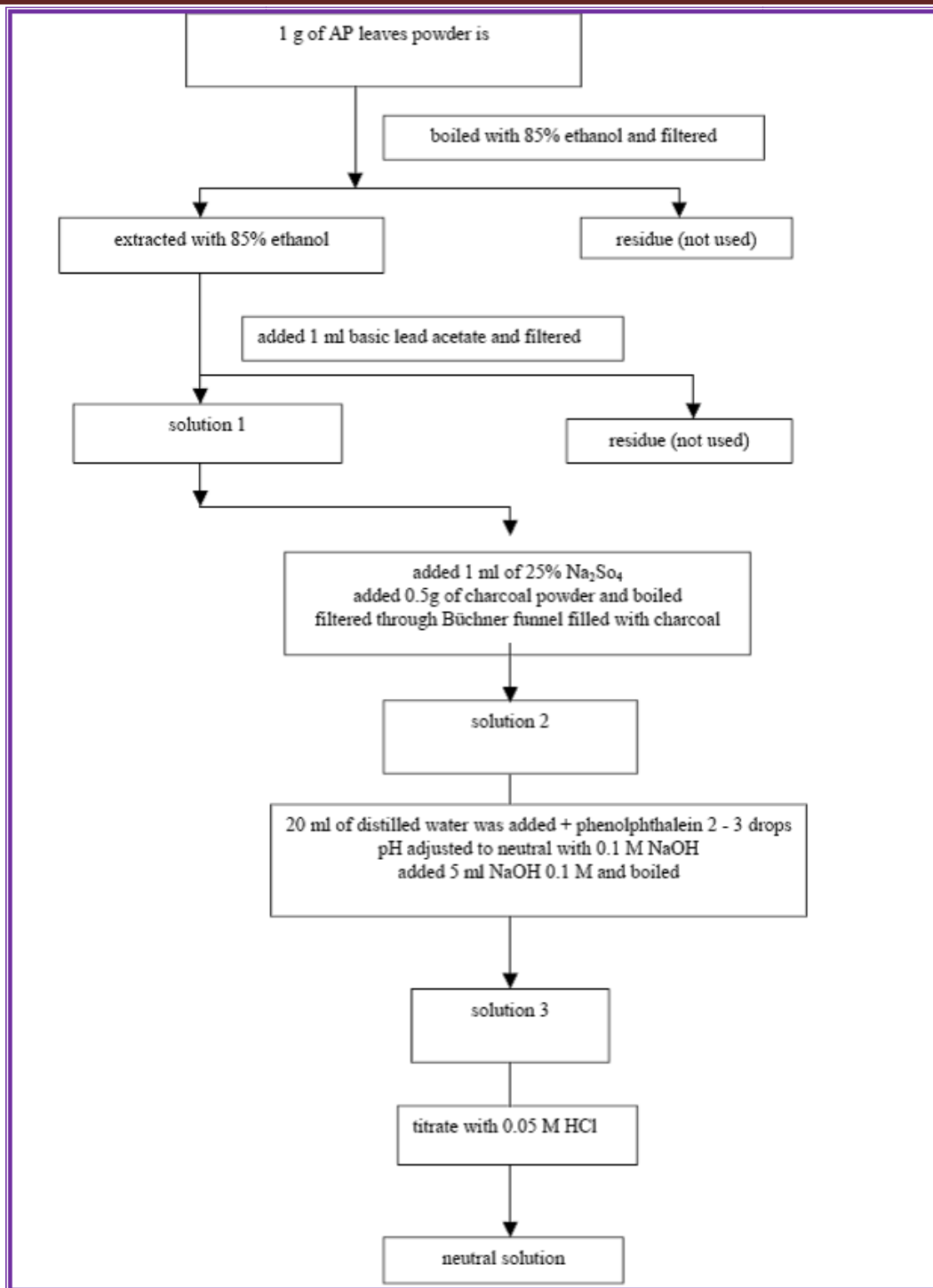


Figure 1. Method to identify the active constituents (diterpene lactones) of AP powder

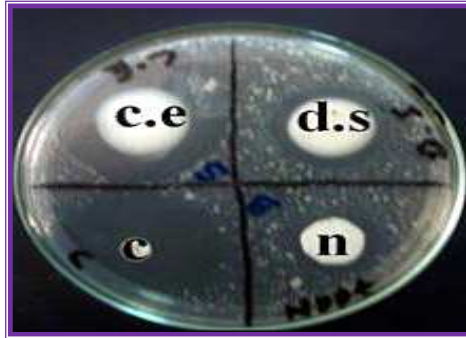


Figure 2. Zone of inhibition of A.niger culture by different andrographolide dilution

C: without andrographolide dilution.

n: filter paper disk dipped in **10** μ l Andrographolide dilution.

d.s: filter paper disk dipped in **20** μ l Andrographolide dilution.

Ce: filter paper disk dipped in **40** μ l Andrographolide dilution.

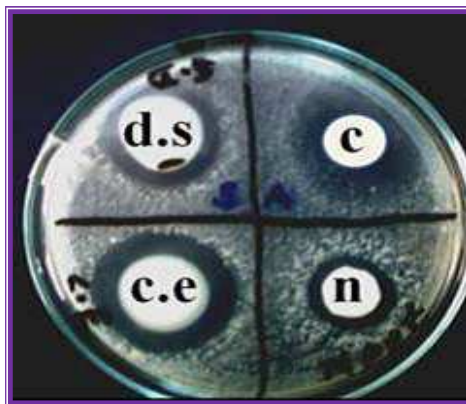


Figure 3. Zone of inhibition of Fusarium by different Andrographolide dilution

C: without andrographolide dilution.

n: filter paper disk dipped in **10** μ l Andrographolide dilution.

d.s: filter paper disk dipped in **20** μ l Andrographolide dilution.

Ce: filter paper disk dipped in **40** μ l Andrographolide dilution.

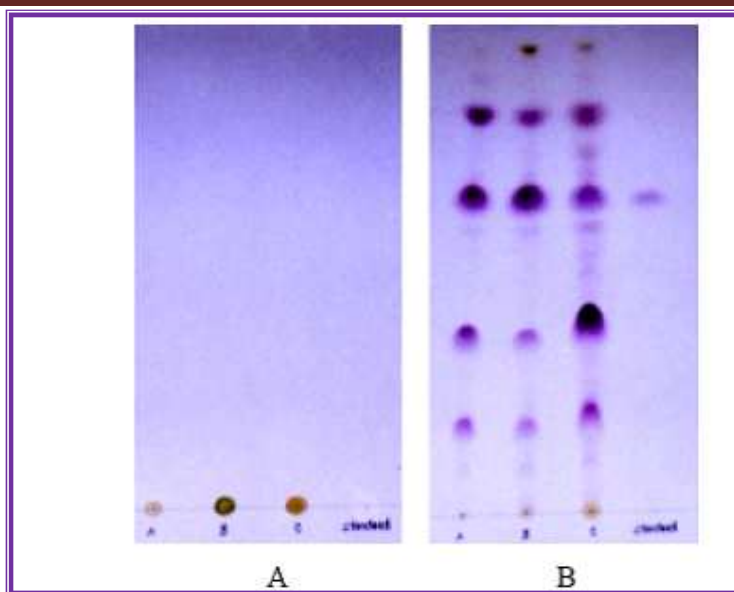


Figure 4. Thin layer chromatography of AP leaves powder before placed in chromatographic tank (A) and after development (B) a - c: AP solvent sample, standard: andrographolide.

Table 1.
Of Chemical Compound

Sr. No	Compounds	Extract of AP leaves
1	Diterpenoids	Present
2	Flavoinds	Absent
3	Glycosides	Present
4	Reducing sugars	Absent
5	Saponins	Absent
6	Steroids	Absent
7	Tannins	Absent
8	Terpenoids	Present

Table 2

Sr. No	Eluant	Fraction no.	No of Fraction collected
1	Hexane	1-10	10
2	EtOAc:Hexane(5:95)	11-15	5
3	EtOAc:Hexane(10:90)	16-25	9
4	EtOAc:Hexane (15:85)	26-40	16
5	EtoAc:Hexane(25:75)	57-62	5
6	EtOAc:Hexane(1:1)	91-102	11
7	EtOAc:Hexane(75:25)	116-125	10
8	EtOAc	141-150	9
9	EtOAc: MeOH(5:95)	151-160	9
10	EtOAc: MeOH(25:75)	161-165	9
11	MeOH	166	Column washed

Table 3.

Of pooled fraction

Name of Pooled Fraction	Fraction no.	Developing solvent system for TLC
A((Trans-phytal)	1-15	Hexane
B	16-33	CHCl ₃ :Hexane(10:90)
C	34-60	CHCl ₃ :Hexane)(10:90)
D	61-90	CHCl ₃ :Hexane(1:1)
E	91-151	CHCl ₃ :Hexane(75:25)
F(14-deoxyandrographolide)	152-158	MeOH:CHCl ₃ (10:90)

Table 4

Sr.No.	Eluant	Fraction No.	No of column washed
1	EtOAc:Hexane(1:1)	1-10	10
2	EtOAc	11-20	10
3	MeOH:EtOAc(5:95)	21-30	10
4	MeOH:EtOAc(25:75)	31-40	10
5	MeOH:EtOAc(1:1)	41-50	10
6	MeOH	51	Column washed

Table 5

Name of pooled fraction	Fraction no.	Developing solvent system for TLC
A	1-15	CHCl ₃ :Hexane(75:25)
B	16-20	MeOH:CHCl ₃ (15:85)
C(Andrographolide)	21-31	MeOH:CHCl ₃ (25:75)
D	32-40	MeOH:CHCl ₃ (35:65)

Table 6.

Thin layer chromatography- confirmatory test

Diterpene lactones	Rf valus	Development(UV254)
Lactone type 1	1-5	-
Lactone type 2	11-15	opacity
Lactone type 3	18-22	opacity
Neoandrographolide	28-32	-
Lactone type 5	49-51	-
Andrographolide	52-56	Opacity
Lactone type7	57-59	Opacity
Lactone type 8	66-68	-
Dehydroandrographolide	69-71	opacity

Table 7

Chemical content of AP

Parameter	AP leaves sample	Standard (%)
Ash value		<20.0
Acid insoluble Ash	1.38	<5.1
Foreign organic matter	0.00	<2.0
Alcohol soluble extractive	17.86	>24.00
Water soluble extractive	22.98	>20.00

Table 8.

Isolate of *Andrographis paniculata*

Sr. No	Compounds	Extract	M.F	M.P (°C)	Identified as
1	AP-1	Ethyl acetate extract	C ₂₀ H ₄₀ O	Viscous	Trans-phytol
2	AP-2	Ethyl acetate extract	C ₂₄ H ₃₄ O ₄	172-173	14-deoxy andrographolide
3	AP-3	Ethyl acetate extract	C ₂₀ H ₃₀ O ₅	220-221	Andrographolide

Table 9.

Inhibition zone

Sr. No	Fungus	Inhibition zone diameter(mm) ^{a,b}		
		10µl(A.S)	20µl(A.S)	40µl(A.S)
1	<i>A.niger</i>	24±0.5	22±0.4	16±0.5
2	<i>F. oxysporum</i>	35±0.5	30±0.5	25±0.5

a: mean value± SD(the zone of inhibition(in mm) including disc of 8mm in diameter)

b: ststistical analysis data are expressed as means± SD

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