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RESEARCH ARTICLE

PHARMACOGNESTIC, PHYTOCHEMICAL, AND ANTIMICROBIAL ACTIVITIES OF NILA VEMBU (*ANDROGRAPHIS PANICULATA.* NEES.)

***Fahad Albahri**

Saudi Arabia

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ABSTRACT

Andrographis paniculata mostly the leaves and roots were used for medicinal purposes. The whole plant is also used in holistic health and longevity, a philosophy and system of healing the whole body, mind and individual. The present investigation was find to carry out the qualitative phytochemicals such as alkaloids, saponins, flavonoids, tannins, phenols, steroids, terpenoids, glycosides, and lipids, quantitative phytochemicals such as alkaloids, saponins, flavonoids, tannins, phenols, steroids, terpenoids, and glycosides, and antimicrobes such as *Aspergillusfumigatus, A.flavous, A.hemicola, A.hemicola, A. terreus, A.niger, Penniciliumcitrinum, Candida albicans, Microsporumepidermophyton* were identified in *Andrographispaniculata* were analyzed. The above all parameters are positively favour for medicinally valuable substances for human and animal environmental activities and behaviours for the natural ecosystem.

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INTRODUCTION

Andrographispaniculata is an extremely bitter in taste in all parts of the plant body. It is also known as Nila-Vembu, meaning "neem of the ground", since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree as *Azadirachtaindica. Andrographispaniculata* mostly the leaves and roots were used for medicinal purposes. The whole plant is also used in some cases. It is qualitative, holistic health and longevity, a philosophy and system of healing the whole body, mind and individual. The leaves contain the highest amount of andrographolide,2.39 the most medicinally active phytochemical in the plant, while the seeds contain the lowest. *Andrographis paniculata* is also used as astringent, bacteria killing agent, painkiller, fever reducer, and treatment for worms. It is an erect annual herb that grows 30 to 110 cm in height and is native to India, China, and Southeast Asia. It is widely cultivated in Asia. The square stem has wings on the angles of new growth and is enlarged at the nodes, while small white flowers with rose-purple spots are borne on a spreading panicle. The plant produces yellowish-brown seeds, and all parts have an extremely bitter taste. Biofertilizers are cost effective, eco friendly and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system in India (Kannaiyan, 2002). It greatly benefits farmers with only very small input cost (Kumudha, 2005; Kumudha and Gomathinayagam, 2007). They have the ability to fix atmospheric nitrogen in soil. Biofertilizers are natural and organic products. They help to provide and keep the soil with all the minerals and microorganisms required for plant growth. Their application is easy, cost effective and does not cause any pollution problem. In India, biofertilizers constitute the best renewable source of nutrient supply to plants and as supplement to chemical fertilizers and organic manures. Biofertilizers are cost effective, eco friendly and renewable source of plant nutrients to supplement chemical fertilizers and organic manures in sustainable agricultural system in India. They are microbial inoculants which enhance crop production through improving the nutrient supplies and their availability (Wani and Lee, 2002). The use of biofertilizers undoubtedly boosted not only the food production but also, it shows the positive effects on physico-chemical properties of soil, nitrogen transformation, macro and micronutrient uptake and nutritional composition (Mahesh and Hosmani, 2004). Among biofertilizers, *Azotobactor* and *phosphobacterium* is recommended for grain legumes and other crop plants to improve productivity and argument the soil nitrogen status. A "good" strain of *Rhizobium Azotobactor* and *phosphobacterium* is capable of forming effective nitrogen fixing nodules in the legumes. These *rhizobia* should be

****Corresponding author:*** **Fahad Albahri,**
Saudi Arabia

superior in their ability to survive in the soil and should have the ability to fix nitrogen symbiotically under diverse agroclimaticconditions (Brahmaprakash and Hudge, 2002).

MATERIALS AND METHODS

Qualitative analysis of the phytochemicals (Mace, 1963; Ramakrishnan *et al.*, 1994)

Qualitative analysis of phytochemicals were carried out in the *Andrograpispaniculata* is an effective medicinal plants.

Test for Tannins: About 0.5 gm. of dried powdered was boiled in 20 ml of water in a test tube and then filtered. A few drops of0.1% Ferric chloride were added to the filterate. Appearances of brownish green colour indicate the presence oftannins.

Test for Saponins: About 2 gm ofthe powdered samples of leaves and stems (both plants) was boiled in 20 ml of distilled water in a water-bath and filtered. The filtrate was mixed with 5 ml ofdistilled water and shaken vigorously for a stable persistent broth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion shows the presence ofsaponins.

Test for Flavonoids: About 5 ml of the diluted ammonia solution was added to a portion of aqueous filtrate of plant extract followed by the addition of concentrated sulphuric acid. Appearance of yellow color shows the presence of flavonoids.

Test for Steroids: Two ml ofacetic anhydride was added to 0.5 ml of ethanolic extract followed by 2 ml Sulphuric acid. The colour changes from violet to green are an indication for the presence ofsteroids.

Test for Terpenoids: Five ml ofthe plant extract was mixed with 2 ml of chloroform and 3 ml of concentrated Sulphuric acid. Formation of reddish brown color at the interface indicates the presence ofterpenoids.

Test for Phenols: Distilled water (2ml) and few drops of 10% ferric chloride were added to 5ml of the plant extract**.** Formation of blue or green color indicates the presence of phenols.

Test for Alkaloids: Three ml ofextract was stirred with 3 ml of 1% HCl on steam bath. 1 ml of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence oforange red precipitated was taken as positive and for the second tube Mayer's reagent was added and appearance of buff colored precipitate was taken as positive test for presence of alkaloids.

Test for glycosides: To 2 ml ofextract with dilute HCl and 2 ml Sodium nitropruside in pyridine and sodium hydroxide solution were added. Formation of pink to blood red color indicates the presence ofCardiac glycosides.

Quantitative analysis of thephytochemicals

Estimation of Alkaloids (Harborne 1973): Five gram ofthe sample was weighed into a 250 ml beaker. 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hours. This was filtered and extract was concentrated on a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract to get precipitation. The whole solution was allowed to settle down and the precipitate was collected and washed with diluted ammonium hydroxide and filtered. The residue was may be alkaloid that was dried and weighed. From this alkaloid content was determine.

Alkaloids Content (%) = B - A × 100 / S

Where,
B = Weight of Whatmann filter paper.
A = Weight of Whatmann filter paper, after drying.
S = Sample weight.

Estimation of Saponins(Obadoni and Ochuko, 2001): 20 g ofsample was placed in a conical flask and added 100 ml of 20% ethanol. The sample was heated over a hot water-bath for four hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over a water-bath at about 90°C. The concentrated extract was transferred into a 250 ml of separator funnel and then 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded and this purification process was repeated. Then 60 ml of n-butanol extract was added. The combined n-butanol extracted was washed twice with 10 ml ofaqueous Sodium Chloride. The remaining solution was heated in a water bath. After evaporation, sample was dried in an oven to a constant weight. The saponins content was calculated as follows.

Saponins content (%) = B – A × 100 / S

Where,

B = Weight of Whatmann filter paper.
A = Weight of Whatmann filter paper with sample.
S = Sample weight.

Estimation of Phenols: 0.5 ml/g ofextract was taken in test tubes. 8 ml ofdistilled water and 0.5 ml ofFolin'sCiocalteau reagent were added to all tubes. The tubes were kept in BOD incubator at 40°C for 10 minutes. Then, 1 ml of sodium carbonate solution was added and the tubes were kept in dark for incubation for an hour. The colour development was read specrophotometrically at 660 nm. Standard graph was drawn using tannic acid as standard. Different concentrations of tannic acid were prepared and OD was read at 660 nm in a Shimadzu UV-1650 spectrophotometer. By ploting the OD value obtained four samples in the standard graph, phenol present in the sample was calculated.

Estimation of Total Flavonoids (Min and Chun, 2005): 100 mg of tannic acid was dissolved in distilled water and the volume was made up to 100 ml. Different concentrations of the standard was obtained by appropriate dilution with distilled water, read at 510 nm and the values were plotted to made standard graph. In a test tube, 0.5 ml of aqueous extract of sample was diluted with 3.5 ml of distilled water at zero time. 0.3 ml of5% sodium nitrate was added to the tube.

After five minutes, 0.3 ml of 10% aluminium chloride was added. At 6th minutes, 2 ml of 1M sodium hydroxide was added to the mixture. Immediately, the content of the reaction mixture was diluted with 2.4 ml of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm about blank. Tannic acid was used as standard compound for quantification of total flavonoids as mg / 100g of edible portion.

Estimation of Tannins: Powdered sample weighing 0.5 gm was transferred to 250 ml conical flask and 75 ml water was added. The flask was heated gently to boil for 30 min. The samples run at 2,000 rpm for 20 min. The supernatant was collected in 100 ml volumetric flask and made up the volume. One ml of the sample was added to 100 ml volumetric flask containing 75 ml water. 5 ml of folin-denis reagent and 10 ml of sodium carbonate solution was added and diluted to 100 ml with water. The mixture was shaking well. Absorbance was read at 700 nm after 30 min. A blank with water instead of the sample was prepared by the absorbance was measured. A standard graph was prepared by plotting the OD value, of known quantity of tannic acid and the tannins content of the sample was calculated as tannic acid equivalent from the standard graph.

Estimation of Steroids (Chanwitheesuk *et al*., 2005): 1ml of extract of different solvents acetone, ethanol, was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20°C for 30minutes with occasional shaking and made up to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank. B-Estradiol is used as a standard material and compared the assay with β-Estradiol (concentration 20 µg) equivalents.

Antimicrobial Activity: For antifungal activity, Potato Dextrose Agar medium were used. Then dispense the media into each of the petridish and allowed it to solidify. Transferred 1ml of 24 hrs fungal culture onto solidified plate and spread it with the help of sterile glass rod. After seeding with fungal culture respectively, made the well at the centre of the media with the help of cork borer. Then transferred different concentration of lycopene to each of the well. Incubated the plates at 37°C for 24 hrs for antibacterial activity and at room temperature for 2 to 4 days for antifungal activity observed plates for zone of inhibition, compared with the control and measure their diameter.

Antifungal activity(Barry and Thomsberry, 1991): Potato Dextrose Agar (PDA) medium was used as the culture medium for fungi. Fifteen ml of sterilized potato dextrose agar medium was poured into each sterile petriplate and allowed to solidify. One ml of 10^6 spore suspension of the fungus was spread over the plate. The sterile filter paper discs (6 mm in diameter) were impregnated with 10 µl of the 3 mg / ml extracts (30 µg / discs) placed on the inoculated agar plates. Negative controls were maintained with the solvents employed for plant extraction and the positive control with Nystatin (30 µg / disc). The plates were incubated at 30°C for 72 hours and the antifungal sensitivity was recorded by measuring the zone of inhibition against the test organisms. The experiments were carried out in triplicates.

RESULTS AND DISCUSSION

Andrographispaniculata is a plant that has been effectively used in traditional aian medicines for countries. *Andrographispaniculata* family Acanthaceae, it is perceived "blood purifying" property results in its use in diseases where bload "abnormalities" are considered causes of disease, such as skin eruptions, boils, scabis, and chronic undetermined fevers. The aerial part of the plant, used medicinally, contains a large number of chemical constituents, mainly lactones, deterpenoidsglycocides. (Yang *et al.,* 2009). *Andrographispaniculata*having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects (Bhatnager*et al.,* 1961). The contribution of nutrients by organic amendments had traditionally been considered to be the best to increase the crop yield. Vermicompost is increased the productivity by maintaining the soil health with concomitant nutrient balance, besides minimizing the pollution hazards as well as fertilizer cost (Gayathri and Anburani, 2008).

The chemical fertilizer is the major supplier of nutrients besides organic manures. The continuous and excess use of chemical fertilizers over a longer period of time has resulted in deterioration of soil health and causes less productivity (Yadav and Lourduraj, 2005a). In this context, the role of organic manures and biofertilizers in sustainable agriculture assumes special significance particularly in the present context of very high cost of chemical fertilizers. Organic farming is becoming a major tool for sustaining the soil quality degraded by intensive use of synthetic chemicals for increasing crop production. Therefore, the use of bio-agents as biofertilizers or biopesticides is an integral part of organic farming. The Vermicompost contain plant growth regulating substances including plant growth hormones and humic acids which are probably responsible for increase in germination, growth and yield of plants (Atiyeh*et al.,* 2002; Aranconе*t al.,* 2006).

Qualitativephytochemicals analysis of *Andrographispaniculata* Nees: Qualitative phytochemical analysis of *Andrographispaniculata*revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and glycosides in both control and treated plants. The identification and isolation of these active compounds could lead to the new drug discovery at acheaper cost which would be useful in medicine.(Savithramma 2011).

Table 1. Qualitative phytochemicals

S.No	Test name	*Andrographispaniculata*(mg/g)	
		Control	Treated
1	Alkaloid	+	+
2	Saponins	+	+
3	Flavonoids	+	+
4	Tannins	+	+
5	Phenols	+	+
6	Sterols	-	-
7	Terpenoids	+	+
8	Glycosides	+	+
9	Lipids	+	+

(+ present, - absent)

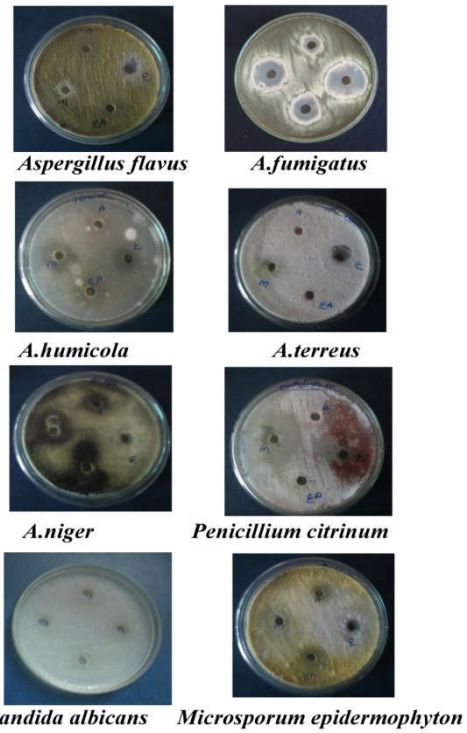
Quantitative phytochemicalsanalysis of *Andrographispanniculata*. Nees: The present study revealed the quantity of different phytochemicals present in control and treated plants. In treated plants contain alkaloids (7.3±1.1), saponins (5.3±0.9), flavonoids (6.4±1.3), phenols (11.54±0.7), terpenoids (7.2±1.3) and glycosides (5.6±1.3) mg/g were recorded. In control plants, the alkaloids content (4.5±1.9), flavonoids content (4.0±2.1), saponins (3.1±1.2), tannins (5.2±2.5), phenols content (8.2±2.5), terpenoids content (5.1±1.9), and glycosides content (2.6±1.2) mg/g of phytochemical compounds were measured.Similar findings were recorded in *Naringicrenulata* plants (Rambabu*et al* 2017). Sarada*et al* (2012) suggested that the leaf and barkextract in *Naringicrenulata* were increased in the phytochemical contents.Similar findings were recorded in *Naringicrenulata* plant leafby Ramachandran*et al* (2010).

Table 2.Quantitative phytochemicals

S.No	Test name	*Andrographispaniculata*(mg/g)	
		Control	Treated
1	Alkaloid	4.5±1.9	7.3±1.1
2	Saponins	3.1±1.2	5.3±0.9
3	Flavonoids	4.0±2.1	6.4±1.3
4	Tannins	5.2±2.5	8.6±0.9
5	Phenols	8.2±2.5	11.54 ± 0.7
6	Sterols	-	-
7	Terpenoids	5.1±1.9	7.2±1.3
8	Glycosides	2.6±1.2	5.6±1.3

(- absent)

Antimicrobial activity of *Andrographispaniculata* Nees.
The antimicrobial activity of *A. paniculata* against some fungi was determined. *A. Paniculata* leaf with ethanolic extract against *A flavus, A.fumigatus, A.terreus, A.niger, Penicilliumcitrinum, P.Crysogenum, Candida albicans, Microsporumepidermophyton* was five, ten, eight, ten, five, eight and five mm.zone of inhibition was recorded respectively.



(E- Ethanol, M- Methanol, EA-Ethyl acetate, A-Aqueous)

Plate I. Antimicrobial activity of different extraction of *Andrographispaniculata* Nees

Whereas in methonolic extract zone of inhibition against *A.terreus*(18mm)was excellent and minimum zone of inhibition was (10 mm) in *Aspergillusand Microsporumepidemophyton* in the case ofethyl acetate zone of inhibition was very less against all respective fungi. The *A.paniculata*with aqeous extract was also only in *A.fumigatus, A.niger*, and *Microsporumepidemophyton* was each 2mm zone ofinhibition was recorded respectively Table 3and Plate I are shown.

Table 3. Antimicrobial activity of *A. paniculata* with different solvents against fungi

S. No	Fungi	Zone of inhibition (mm)			
		Ethanol	Methanol	Ethyl acetate	Aqueous
1	*Aspergillus.flavus*	5	10	-	-
2	*A.fumigatus*	10	15	3	-
3	*A.humicola*	8	12	2	2
4	*A.terreus*	10	18	3	-
5	*A.niger*	5	15	2	2
6	*Penicilliumcitrinum*	-	14	-	-
7	*Candida albicans*	8	15	2	-
8	*Microsporumepidermophyton*	5	10	3	3

(- absent)

Conclusion

Andrographispaniculata has been used in Ayurvedha, unani, and Siddha system ofmedicines from all over the world. The plants were given either in the form ofpowder extracts are in its isolated compounds are fortified has been used in national and international markets for various diseases. These are safe nontoxic and strong natural antioxidant potential and well known for its medicinal properties and widely used by oriental countries. Bio fertilizers are natural and nontoxic beneficial microbes in soil and environments. It is ecofriendly and improves plant growth and productivity of medicinal plants and benefit for our nation.

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