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PROCEEDINGS

"Globalization , Entrepreneurship and Emerging Trends of New Aspects of Higher Education in India"

4th-5th April 2024

Organized by

St. Columba's College, Hazaribag
(A Constituent unit of Vinoba bhave University, Hazaribag)

In Association with

Vinayam Research Association
(Regd., under MSME) Branch of Institution-Dhanbad, Jharkhand

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EDITOR DR. BINAY KUMAR SINGH

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Phytoactive Assessment of Medicinal Plants Inured by the Aboriginal Dwellers from Surajpur District of Chhattisgarh

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Abstract

The medicinal plants are endowed with a tremendous opulence of bioactive phytochemicals as curatives for diverse ailments since time immemorial. The Surajpur district possess immense diversity of medicinal flora with proven expeditious ameliorative efficacy by the aboriginal inhabitants. Owing to this present scenario, the current investigation pertains to the qualitative and quantitative estimation of phytochemicals from the leaves of *Andrographis paniculata*, *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes*. At the very onset, powdered leaves samples of the aforesaid medicinal plants were extracted in petroleum ether and aqueous according to their polarity index in the soxhlet apparatus. Subsequently, the qualitative exploration of primary metabolites *viz*., carbohydrates, protein and starch revealed their presence more so in aqueous than petroleum ether extracts of *Andrographis paniculata* followed by *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes*. However, the qualitative examination of secondary metabolites such as alkaloids, flavonoids, glycosides, steroid, terpenoids, saponin and phenol revealed their predominant presence more so in aqueous than petroleum ether extracts of *Andrographis paniculata*, *Vitex negundo* and *Aegle marmelos*. However, their presence was recorded more in petroleum ether than aqueous extracts of *Pistia stratiotes*. Eventually, the quantitative analysis of flavonoid in the leaves samples divulges its presence more in case of *Andrographis paniculata* followed by *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes* respectively. The aforementioned repercussions distinctly spots light on the enormous potentiality of phytoactives from the medicinal plants under inspection. In time ahead cutting-edge efforts will be anticipated to extract the bioactive compound from these medicinal plants and to elucidate the complete structure of phytoactives conferring specific bioactivity using several sophisticated spectral instruments. The present research will definitely lay the strong foundation of herbal therapeutics and certainly contribute for the better, safer and cost-effective novel drug development in near future.

Keywords: Phytoactive, Soxhlet, Qualitative, Quantitative, Flavonoid, Polarity Index

Introduction:

Medicinal plants are the richest bio-resource of diverse primary and secondary metabolites as a vital source of nature's boon for curing several ailments since long back. The plant based herbal therapy is widely explored in the traditional system of medicine and their curative potentials are well documented (Dubey *et al*., 2004; Pandey and Gupta, 2019). Thus, the documentation of the plants phytochemicals to treat and prevent diseases has attracted the attention of scientist's worldwide (Falodun *et al*., 2006). Their curative potentiality is due to the presence of a wide array of complex chemical compounds known as secondary metabolites present in different parts of medicinal plants namely root, stem and leaf (Jalapure *et al*., 2004; Lewis *et al*., 206). The phytoactive principles of medicinal plants possess secondary metabolites such as alkaloids, flavonoids, glycosides, steroid, terpenoids, saponin and phenol (Arokiyaraj *et al*., 2009; Kumar *et al*., 2010). The medicinal properties of plants are due to their antioxidant, antimicrobial, antipyretic, anti-inflamatory and antitumour activity of the phytochemicals present in them (Gantait *et al*., 2011). There is, therefore, urgent need to look for the search of efficacious medicinal plants with the aim of validating their ethnomedicinal importance and subsequently the isolation, purification and characterization of bioactive compounds which will be added to the potential list of drugs in near future.

Surajpur is a district located in northern Chhattisgarh, India. The administrative headquarter of Surajpur district is Surajpur city. It was selected as the study site for the current investigation since it is one of Chhattisgarh's tribally prosperous districts. The coordinates of Surajpur are 23.223047 latitude and 82.870560 longitude. 23° 13' 22.9692" N and 82° 52' 14.0160" E are its GPS coordinates. Six tehsils, namely Bhaiyathan, Odagi, Pratappur, Premnagar, Ramanujnagar, and Surajpur, constitute the Surajpur district (Fig. 1). Along with its abundant natural resources, Surajpur is surrounded by foreste, ancient temples including the Bhageshwari Devi, Durga, Mahamaya, Patal Bhairav, and Shyam Baba temples are located in the nearby area of interest. A pleasant and moderate environment, with the winter months typically lasting from November to February, is a gift of nature to the Surajpur district. The summer season, however, runs from March through June. Central India is located along the tropic of cancer, which causes a rise in temperature of up to 46°C. Rainfall in this area ranges from 1000 to 1050 mm, and it is driven by south-western disturbances in the Arabian Sea.

Fig. 1: Location of Surajpur district in Chhattisgarh, India

Materials and Methods:

Selection of Medicinal Plants:

The present research was carried out in Surajpur district of Chhattisgarh. The leaves of *Andrographis paniculata* (Family-Acanthaceae), *Aegle marmelos* (Family-Rutaceae), *Vitex negundo* (Family-Lamiaceae) and *Pistia stratiotes* (Family-Araceae) were selected based on its traditional usage by the tribal community of Surajpur district of Chhattisgarh in curing several ailments and its ethno-medicinal importance as herbal drug. Apparently healthy and diseased free medicinal plants were selected for the phytochemical analysis.

Collection of the Samples:

The fresh leaves of *Andrographis paniculata*, *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes* were collected, identified and their herbarium was also prepared and preserved at the department of Botany, Govt. Rewati Raman Mishra P.G. College, Surajpur-497229, Chhattisgarh, India. The leaves samples were washed under running tap water to remove debris and shade dried for about three weeks to attain a constant weight. The dried samples were mechanically grinded by using a mortar and pestle and finally powdered by laboratory blender (Remi) and stored in separate air tight bottles till use (Fig. 2).

(a) *Andrographis paniculata*

(b) *Aegle marmelos*

(c) *Vitex negundo*

(d) *Pistia stratiotes*

Fig. 2: Medicinal Plant Samples (a) *Andrographis paniculata*, (b) *Aegle marmelos*, (C) *Vitex negundo* and (d) *Pistia stratiotes*

(a) *Andrographis paniculata*: *A. paniculata* is an annual, branched herbaceous plant erecting to a height of 30-110 cm in moist shady places with stem acutely quadrangular, much branched, fragile, leaves are simple opposite and lanceolate.

(b) *Aegle marmelos*: It is a slow growing, medium sized tree, up to 12-15 m tall with short trunk, thick, soft and spreading, spiny branches, deciduous, alternate leaves, borne singly or in group are composed of 3-5 oval, pointed toothed leaflets, long petiole.

(c) *Vitex negundo*: *V. negundo* is a woody, erect and large aromatic deciduous shrub which grows to small tree of height 2-5 m with quadrangular branches. The leaves are pentafoliate and leaflets are arranged palmately and terminal leaflet.

(d) *Pistia stratiotes*: *P. stratiotes* also known as jalkumbhi. It is a perennial and floats on the surface of water root hanging submerged beneath floating leaves. The leaves are green with 2-5 cm long.

Extraction Procedure:

(a) Extraction by Water Bath:

15 g powdered leaves samples were extracted with 150 ml of aqueous and petroleum ether in the beaker according to their polarity index. The thimble was carefully placed inside the beaker containing respective organic solvents and the beaker was covered by watch glass. Above the watch glass another beaker containing ice cubes were placed to furnish the process of condensation. After extraction, the concentrated extracts so obtained were further dried in incubator at 40^{o}C. The residual extracts after drying were stored in refrigerator at 4^{o}C in small and sterile glass tubes (Fig. 3).

Fig. 3: Extraction of Phytochemicals in Water Bath

(b) Extraction by Soxhlet Apparatus:

15 g powdered leaves samples were extracted with 150 ml of aqueous and petroleum ether according to their polarity index for 8-10 h in the soxhlet apparatus (Tempo) at a temperature not exceeding the boiling point of the respective solvents. After extraction excess solvent was removed by distillation and the concentrated extracts so obtained were further dried in incubator at 40^{o}C. The residual extracts after drying were stored in refrigerator at 4^{o}C in small and sterile glass tubes (Fig. 4).

Fig. 4: Extraction of Phytochemicals in Soxhlet Apparatus

Qualitative Phytochemical Analysis of Primary Metabolites:

1. **Carbohydrate Test:**

(a) Benedict Test: 2 ml test solution was taken in a test tube and added 3 ml benedicts solution and boiled. The appearance of brown red or rust colour precipitate indicates the presence of carbohydrates.

(b) Fehling's Test: 2 ml test solution was taken in a test tube and added Fehling's solution and boiled. The appearance of brownish red colour indicates the presence of carbohydrates.

2. **Protein Test:**

(a) Biuret Test: 2 ml test solution was taken in a test tube and added 2-3ml biuret reagent. The solution turns into purple after half an hour, indicates the presence of protein.

(b) Xanthoprotic Test: 2 ml test solution was taken in a test tube and added 1 ml concentrated HNO_3, the white precipitate is obtained which turns yellow upon heating and thereafter adding concentrated NaOH the yellow color turns into orange color, indicating the presence of protein.

3. **Starch Test:** 2 ml test solution was taken in a test tube and 1ml iodine solution was added the appearance of blue colour indicates the presence of starch.

Preparation of Reagents/Solutions:

1. **Preparation of Mayer Reagent:**

   The reagent is prepared by dissolving a mixture of mercuric chloride (1.36 g) and potassium iodide (5 g) in 100 ml water.

2. **Preparation of Wagner's Reagent:**

   This reagent is prepared by dissolving 2 g of iodine and 6 g of potassium iodide in 100 ml of distilled water.

3. **Preparation of Ferric Chloride Reagent:**

   This reagent is prepared by dissolving 1.22 g of ferric chloride in 100 ml distilled water.

4. **Preparation of Lead Acetate Reagent:**

   This reagent is prepared by dissolving 2.88 g of lead acetate in 100 ml of distilled water.

5. **Preparation of Biuret Reagent:**

   This reagent is prepared by dissolving 0.5% $CuSo_4$ in 20% KOH.

Qualitative Phytochemical Analysis of Secondary Metabolites:

Qualitative phytochemical analysis of secondary metabolites from leaves extract of medicinal plants was carried out following (Harborne, 1973; Trease and Evans, 1989)

1. Test for Alkaloids:

(a) Mayer's Test: 2 ml test solution was taken in a test tube and added 2 ml dilute HCl and few drops of Mayer's reagent formation of creamy white precipitate indicate presence of alkaloids.

(b) Wagner's Test: 2 ml test solution was taken in a test tube and added 2 ml 2% H_2So_4 then boiled and added few drops of Wagner's reagent formation of reddish-brown precipitate indicates the presence of alkaloids.

2. Test for Flavonoids:

(a) Ferric Chloride Test: 2 ml test solution was taken in a test tube and added few drops of ferric chloride the appearance of blackish red colour indicates the presence of flavonoid.

(b) Lead Acetate Solution Test: 2 ml test solution in a test tube was treated with few drops of 10% lead acetate solution formation of yellow precipitate indicates the presence of flavonoids.

3. Test for Glycosides:

(a) Keller-Killiani Test: 1ml test solution in a test tube was treated with 2 ml glacial acetic acid and added few drops of 5% ferric chloride solution and 1 ml concentrated H_2So_4, the brown ring at interface indicates the presence of glycosides.

(b) Bromine Water Test: 2 ml test solution was taken in a test tube and added 2 ml bromine water, the formation of yellow precipitation indicates the presence of glycoside.

4. Test for Steroids:

(a) Liberman-Burchard Reaction: 2 ml test solution in a test tube was treated with few drops of chloroform and concentrated H_2So_4 was carefully added to form a lower layer. A reddish-brown color at the interface indicates the presence of steroids.

5. Test for Terpenoids:

(a) Salkowski Test: 0.5 ml test solution in a test tube was taken and added 2 ml chloroform and 1 ml concentrated H_2So_4, the color turned into red brown at the interface indicates the presence of terpenoids.

6. Test for Saponin:

(a) Foam Test: 1 ml test solution in a test tube was mixed with 2 ml distilled water and shaken for 5 min. and observed the formation of froth, indicating the presence of saponin.

7. Test for Phenols:

(a) Ferric Chloride Test: 1ml, test solution in a test tube was treated with 1 ml, 5% ferric chloride solution, the appearance of blue-black precipitate indicates the presences of phenol.

Quantitative Phytochemical Analysis of Secondary Metabolites:

Determination of Total Flavonoid Content (TFC):

10 g of sample was weighed in a 250 ml titration flask and 100 ml of the 80% aqueous methanol was added at room temperature and shaken for 4 h in an electric shaker. The entire solution was filtered through Whatman filter paper No. 42. The process was repeated. The whole filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed as total flavonoids (Boham and Kocipai, 1994).

Result and Discussion:

The present research deals with the qualitative and quantitative estimation of phytochemicals from the leaves of four medicinal plants *viz*., *Andrographis paniculata*, *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes*. The results of qualitative exploration of primary metabolites such as carbohydrates, protein and starch revealed their presence more so in aqueous than petroleum ether extracts of *Andrographis paniculata* followed by *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes* (Table 1).

The qualitative examination of secondary metabolites such as alkaloids, flavonoids, glycosides, steroid, terpenoids, saponin and phenol revealed their predominant presence more so in aqueous than petroleum ether extracts of *Andrographis paniculata*, *Vitex negundo* and *Aegle marmelos*. However, their presence was recorded more in petroleum ether than aqueous extracts of *Pistia stratiotes* (Table 2). The results of the above analysis revealed variations in phytoconstituents in different medicinal plants and solvents which might be responsible for their bioactivity (Ramalingam *et al*., 2010; Firdouse and Alam, 2011).

Table 1: Qualitative Analysis of Primary Metabolites from Leaves of Medicinal Plants

Primary Metabolites Test	***A. paniculata***		***A. marmelos***		***V. negundo***		***P. stratiotes***	
	AQ	**PE**	**AQ**	**PE**	**AQ**	**PE**	**AQ**	**PE**
Carbohydrate								
Alpha Naphthol Test	+	-	+	-	-	-	+	+
Benedict Test	+	+	+	-	+	-	-	-
Fehling Test	+	+	-	-	+	+	-	-
Protein								
Biuret Test	+	-	+	-	-	-	-	-
Xanthoproteic Test	+	+	+	-	+	-	-	-
Starch								
Iodine Test	+	+	+	-	-	-	-	-

(+ Positive; - Negative; AQ = Aqueous; PE = Petroleum Ether)

Table 2: Qualitative Analysis of Secondary Metabolites from Leaves of Medicinal Plants

Secondary Metabolites Test	***A. paniculata***		***A. marmelos***		***V. negundo***		***P. stratiotes***	
Alkaloids	**AQ**	**PE**	**AQ**	**PE**	**AQ**	**PE**	**AQ**	**PE**
Mayer's Test	-	-	-	-	-	-	-	+
Wagner's Test	+	+	+	+	+	+	-	+
Flavonoids								
Ferric Chloride Test	-	-	-	-	+	-	-	+
Lead Acetate Test	+	+	-	+	+	-	+	+
Glycosides								
Keller-Killiani Test	+	-	+	-	-	-	-	+
Bromine Water Test	-	+	+	-	+	+	-	-
Steroid - Liberman Burchard Test	+	-	+	-	+	-	-	-
Terpenoids - Salkowski Test	+	+	+	-	+	+	-	-
Saponin - Foam Test	+	-	+	-	-	+	+	-
Phenol - Ferric Chloride Test	+	+	-	-	+	-	-	-

(+ Positive; - Negative; AQ = Aqueous; PE = Petroleum Ether)

Eventually, the quantitative analysis of flavonoid in the leaves samples divulges its presence more in case of *Andrographis paniculata* followed by *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes* respectively (Fig. 5). Findings depicting the higher amount of flavonoids were also documented by several researchers (Sathya *et al*., 2013; Dutta, 2015). Flavonoids are hydroxylated phenolic substances synthesized by the plants in response to microbial infection and have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall leading to cell death (Marjorie, 1999). The aforementioned repercussions distinctly spots light on the enormous potentiality of phytoactives from the medicinal plants under inspection. In time ahead cutting-edge efforts will be anticipated to extract the bioactive compound from these medicinal plants and to elucidate the complete structure of phytoactives conferring specific bioactivity using several sophisticated spectral instruments. The present research will definitely lay the strong foundation of herbal therapeutics and certainly contribute for the better, safer and cost-effective novel drug development in near future.

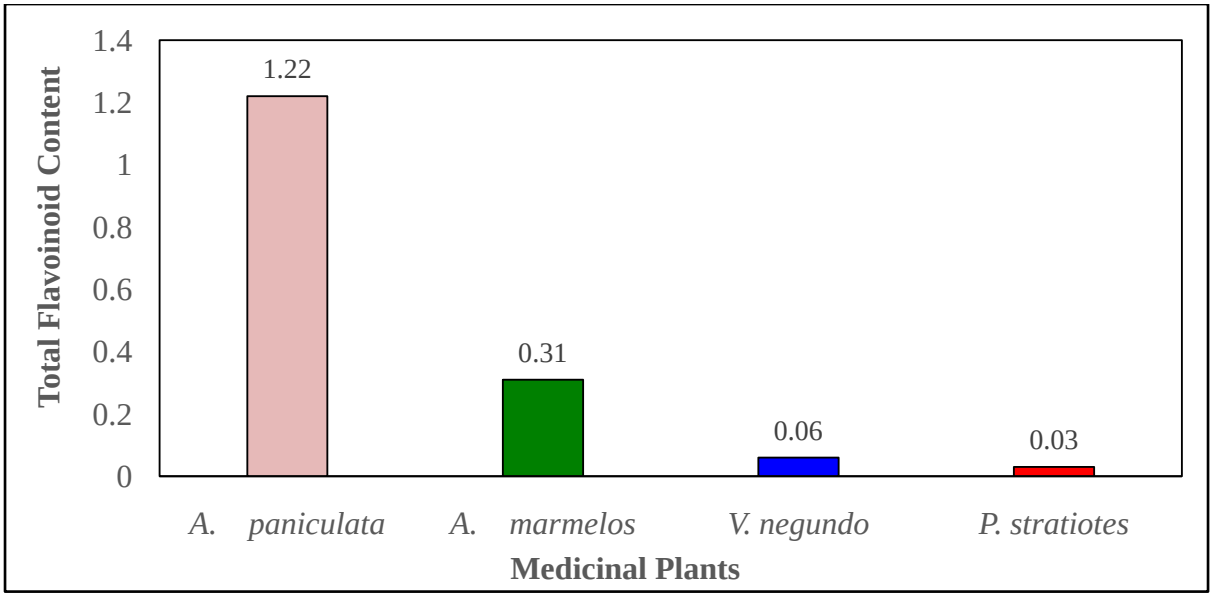

Fig. 5: Quantitative Analysis of Flavonoid from Leaves of Medicinal Plants

Conclusion:

The present investigation was carried out to explore the phytoactive assessment of medicinal plants accustomed by the local inhabitants from Surajpur district of Chhattisgarh. The results were promising and revealed that all the four medicinal plants under study possess a rich abundance of primary metabolites *viz*., carbohydrates, protein and starch as well as secondary metabolites such as alkaloids, flavonoids, glycosides, steroid, terpenoids, saponin and phenol in their leaves. Moreover, the quantitative analysis of flavonoid in the leaves samples divulges its presence more in case of *Andrographis paniculata* followed by *Aegle marmelos*, *Vitex negundo* and *Pistia stratiotes* respectively. However, the present study is a primary platform to explore local potential medicinal plants possessing enormous abundance of phytoactive compounds possessing curative potentiality. Moreover, further purification of these crude extracts from medicinal plants and their chemical characterization using sophisticated analytical instruments such as UV-Visible spectroscopy, High Performance Liquid Chromatography, FT-IR, NMR and ESI-MS could be the future direction of this research, which will definitely pave the path for phytotherapy in future medicine.

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About College

One of the oldest colleges with provision for higher education in Jharkhand and across eastern India, St.Columba's College, Hazaribagh owes its name to the esteemed Irish Saint Columba. St.Columba's College is accredited by UGC and has been affiliated to the Vinoba Bhave University since 1992. The institution outreaches on a public domain and offers every fortune to the students of major disciplines of Arts and Science. Dispersed over a sweeping area of 23 acre campus, the college hosts a multifarious range of facilities to provide ample aid in delivering quality and efficacy and undoubtedly flatters itself to be one of the best colleges in Jharkhand.Students desiring to be future ready definitely have to look for not one, but a whole gamut of conditions to pave their way to a notable as well as exemplary career path.

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