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Full Length Research Article

Comparative Preliminary Qualitative Phytochemical Determination of Medicinal Plants of Uttarakhand

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Abstract

The medicinal functions shown by various different medicinal herbs are mainly due to the presence of numerous phytochemicals i.e., are plant pigments having a number of natural therapeutic functions. The colour qualitative screenings of such pigmented phytochemicals are expressed as the major vital sources for many phenols, flavonoids, tannins, terpenoids, alkaloids helping in treatment of various destructive and life-threatening diseases. It is very much essential to understand and identify the phytochemical bioactive compounds found in many medicinal plants. In recent study, ethanol and methanol plants root extraction were performed of five medicinally important herbal plants which are *Withania somnifera* (Ashwagandha), *Terminaliya arjuna* (Arjuna), *Bacopa monnieri* (Brahmi), *Ranunculus sceleratus* (Jaldhaniya) and *Acalypha indica* (Kuppi) by maceration plant extraction technique. Preliminary qualitative analysis was done of each five each ethanol and methanol extracts and comparative analysis was done. Various tests containing saponins, coumarins, proteins, phenols, anthraquinones, tannins, flavonoids, steroids-sterols, terpenoids, carbohydrates, alkaloids, glycosides were analyzed qualitatively using the most standard optimised protocols. Investigation showed high amounts of all bioactive compounds were present in ethanol and methanol extracts of *Withania somnifera, Terminaliya arjuna* and *Acalypha indica*. It contained a very high levels of phenols, flavonoids, alkaloids, terpenoids, tannins, saponins. Whereas *Bacopa monnieri* and *Ranunculus sceleratus* had shown a very significantly low amounts of phytochemicals. Anthraquinones, glycosides, phenols were found to be very less. Therefore, different pigmented phytochemicals have a diverse extensive range of medicinal activities, enhancing the biological immune system, providing high resistance against infectious diseases in order to protect the host body.

***Key words*:** Medicinal plants, Plant pigments, Phytochemicals, Root extracts, Phenols, Flavonoids

Medicinal plant importance is mainly in drug discovery, identification and development against various diseases since human history [1-2]. Researchers had been guided by traditional folk therapies using wild plants species for novel treatments for developing a healthier life [2-4]. Phytomedicines in drug discovery practice, are basic tools in government health organization department of India. Bioactive secondary natural products are the molecules created by evolution containing physico-chemical properties and are highly potential drug possibilities than those synthetic manmade products formed by fusion of chemical methods [5]. These protocols possess a danger to ecological habitat, environmental disturbance and threatens species survival [6-7].

There are certain medicinal plants which are needed to get scientifically evaluated. The main purpose of this study is to pinpoint the major bioactive constituents occur in the plants of India. Himalayas Uttarakhand and many regions of Uttar Pradesh are the natural flora and fauna having huge resource of medicinal herbs with many reserves mixed scale of biodiversity hotspots accounting highest collections medicinal plant species habitat and uniqueness [8]. Because of growing therapeutic interest in the medicinal plants characterizations in terms of pharmaceutical industry, it is well important for continuing investigation for the identification of many potential bioactive substances from the root parts of *Withania somnifera* (Ashwagandha), *Terminaliya arjuna* (Arjuna), *Bacopa monnieri* (Brahmi), *Ranunculus sceleratus* (Jaldhaniya) and *Acalypha indica* (Kuppi). These herbs are very crucial for maintaining livestock and health. Therefore, the phytochemical screening of novel plants are of huge step [9]. It had been researched that these five herbs contain vast amounts of beneficial secondary metabolites with great amounts of phenolic compounds. As the plant derived potent drugs demands increases, their safe levels increases than synthetic drugs [10-12].

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It has been reported in India, that phytochemicals present in Himalayan regions of plants reserves are majorly responsible for curing a number of diseases and promoting better health, lifestyle [10]. These herbs had been studied deeply to create their proper efficacy, understanding their therapeutic working mechanisms along each of their multiple actions [10-11]. Such important studies had incorporated certain steps like discovery, identification, isolation of those bioactive components, establishing *in vitro* and *in vivo* evaluation of their excessive potency in many experimental animal models and also in epidemiological, clinical-case limited studies in human beings [12]. Many study findings suggested that phytochemicals helps in reducing the risk of heart diseases like coronary heart diseases by blocking the low density lipoprotein (LDL) cholesterol oxidation, cholesterol synthesis as well as absorption reduction, blood pressure normalizing and removing clotting problems, boosting arterial elasticity [12]. Phytochemicals mainly phenols and flavonoids being the major classes detoxify harmful compounds that causes cancer. These bioactive compounds basically neutralize free radicals causing free radicle chain reaction at molecular level, inhibiting various enzymes that activates carcinogens with other enzymes detoxifying carcinogens [13]. Many physiologic properties of phytochemicals are needed to get understand and now a days researchers have been focusing on their vital roles in prevention and treating of cancer and brain-heart disease [13-14]. These pigmented compounds are being promoted for their presence of phenols and flavonoids as it helps in prevention of harmful infections, life term diseases, diabetes, nervous damage, high blood pressure, brain problems, macular degeneration etc., [15]. While these phytochemicals are recognized by their different functions, an individual specific compound may exhibit many biological therapeutic functions [15].

MATERIALS AND METHODS

Plant root collection and authentication

In this experiment, the plant roots were fetched from Agrakhal and Surajpur in Uttarakhand and Uttar Pradesh respectively. Those roots were stored in air tight plastic container from the area. The roots were grinded, washed with hot water (60 °C) and sun dried. Further proper labelling was done. The plants were authenticated through Dr. Swapnal Sisodia, a plant specialist, Subodh College, Jaipur, in Rajasthan, India.

Root extraction: Alcoholic plant mixtures were formed by adding each 5-root powder and solvents i.e., analytical grade 100% ethanol and methanol in 1:10 concentration respectively. 2-5 g of root material was weighted and mixed with 200-500ml of solvents in a brown bottle. It was completely air packed by aluminium foil and placed in shaker for 3-5 days in laboratory at specific room temperature (25-27 °C). The solution was boiled at around 50-60 °C and filtered using 3 muslin cloth layers and then using Whatman No. 1 filter paper. The filtrate was air dried and stored in stopper glass bottle at 4 °C [9].

Phytochemical evaluation

Total 10 phytochemical tests were done by standardized methods [9-11].

(a) *Flavonoids test*: 1ml of root extract was treated with 2-4 drops of concentrated 2N 0.1% sodium hydroxide solution. Yellow colour formation, indicated the presence of flavonoids, which was disappeared by 1-3 drops of concentration sulphuric acid to colourless solution.

(b) *Saponins test*: 1ml of root extract was diluted by 5ml of distilled water and was shaken continuously for 5-10 minutes. The froth appearance indicated presence of saponins.

(c) *Alkaloids test*: 1ml of root extract was treated with 5-6 drops of 0.1% mercuric chloride and potassium iodide solution. The yellow colour precipitate indicated alkaloids presence.

(d) *Phenols test*: 1ml of root extract was treated with 3-5 drops of 0.1% ferric chloride solution. Appearance of brownish, black or greenish blue colour indicated the presences of phenols.

(e) *Tannins test*: 1ml of root extract was treated with 2-4 drops of 0.1% ferric chloride solution. Appearance of brownish, black or greenish blue colour indicated tannins presence.

(f) *Anthraquinones test*: 1 ml of root extracts were treated with 1 ml of 1% benzene solution and 1ml of 1% ammonia solution. A red colour precipitation indicated anthraquinones presence.

(g) *Glycosides test*: 1 ml of root extract was treated with 1 ml of concentrated 1N sulphuric acid and incubated for 2 min. Reddish colour precipitation confirmed glycosides presence.

(h) *Steroids-sterols test*: 1ml of root extract was treated with 2 ml of acetic acid followed by concentrated 2-4 drops of 1N sulphuric acid. Violet, blue or bluish-green colour confirmed the presence of a steroids and yellow colour confirmed presence of sterols.

(i) *Carbohydrate test*: 1ml of root extract was treated with 5-6 drops of Molisch's reagent followed by 1 ml of concentrated 1N sulphuric acid. The solution was incubated for 2 min and diluted with 2ml of distilled water. Appearance of red or violet colour at the two interphase layers confirmed carbohydrates presence.

(j) *Terpenoids test*: 1ml of root extract was treated with 1 ml of ethanoic anhydride followed by 3-4 drops of concentrated 1N sulphuric acid. Colour change from pink to dark violet confirmed terpenoids presence.

(k) *Protein test*: 1ml of root extract was treated with 4-5 drops of 0.1% ninhydrin solution. A dark violet-purple colour confirmed the amino acid presence.

(l) *Coumarins test*: 1ml of root extract was treated 0.2ml of 0.1% 2N sodium hydroxide solution and then 1ml of chloroform. Yellow colour formation indicated coumarin presence.

RESULTS AND DISCUSSION

Root part extraction

The ethanolic and methanolic root extraction of five plants of *Withania somnifera*, *Terminalia arjuna*, *Bacopa monnieri*, *Ranunculus sceleratus* and *Acalypha indica*. Maceration method has been performed for extracting major classes of bioactive (pharmacologically active) compounds from the root part. It had been noted that many different light and dark diverse range of pigmented compounds had been extracted and showed in (Fig 1). Each root extracts colours with respect to the particular solvent used had been contributing to

the number potential therapeutic compounds, indicating significantly high presence of these various components, containing beneficial therapeutic properties.

Preliminary qualitative evaluation

The qualitative screening of 5 roots plants extracts using 2 different solvents i.e., ethanol and methanol had been depicted in (Table 1-2) respectively. It was revealed that the higher classes of valuable phytochemical pigmented compounds were present, based on the different intensities of different root extracts colours with respect to each solvent.

It was noted that both alcoholic extracts of *Withania somnifera*, *Terminalia arjuna* and *Acalypha indica* had a presence of higher levels of terpenoids, proteins, saponins, flavonoids, tannins, coumarins, phenols, carbohydrates, anthraquinones etc depicting great potential medicinal values. These root extracts had a huge broad scale of bioactive components in nature with significant higher ratio of precipitation (++) and (+++). Ethanolic and methanolic root extracts of *B. monnieri* detected compounds in a very low amount (+) except carbohydrates, tannins, saponins and steroid-sterols which were present moderately (++). Ethanolic and methanolic root extracts of *R. sceleratus* resulted to had very low concentration of major potential compounds except saponins (++).

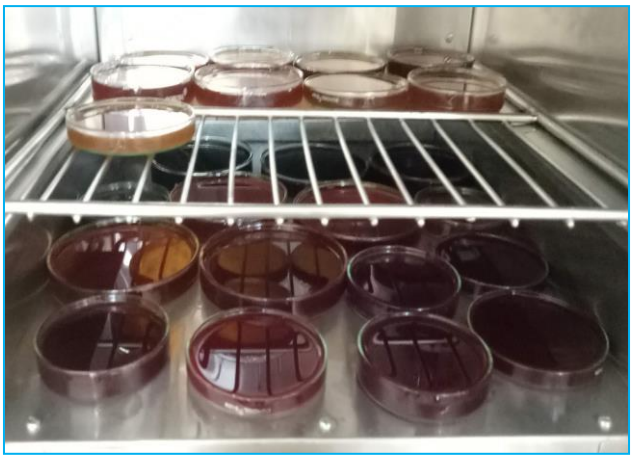

Fig 1 Plant root extraction plates

Table 1 Phytochemical determination of ethanolic extracts

S. No.	Bioactive tests	Plant root extracts				
		W. somnifera Ethanolic extract	*T. arjuna* Ethanolic extract	*B. monnieri* Ethanolic extract	*R. sceleratus* Ethanolic extract	*A. indica* Ethanolic extract
1.	Alkaloids	+++	+++	+	+	++
2.	Tannins	+++	++	+	+	+++
3.	Phenols	+++	+++	+	+	+++
4.	Carbohydrates	++	+++	++	+	+++
5.	Flavonoids	+++	+++	+	+	++
6.	Triterpenoids	+++	+++	+	+	++
7.	Saponins	++	++	++	++	+++
8.	Steroids- Sterols	++	+++	++	+	+++
9.	Anthraquinones	++	+++	+	+	++
10.	Glycosides	++	+++	+	+	+++
11.	Coumarins	++	++	+	+	+++
12.	Proteins	++	+++	+	+	++

(-) Absent, (+) Present, (++) Moderate Amount, (+++) High Amount

Table 2 Phytochemical determination of methanolic extracts

S. No.	Bioactive tests	Plant root extracts				
		W. somnifera Methanolic extract	*T. arjuna* Methanolic extract	*B. monnieri* Methanolic extract	*R. sceleratus* Methanolic extract	*A. indica* Methanolic extract
1.	Alkaloids	+++	++	+	+	++
2.	Tannins	+++	++	++	+	+++
3.	Phenols	+++	+++	+	+	+++
4.	Carbohydrates	+++	++	++	+	+++
5.	Flavonoids	+++	+++	+	+	+++
6.	Triterpenoids	+++	+++	+	+	+++
7.	Saponins	+++	+++	+	++	+++
8.	Steroids- Sterols	+++	+++	++	+	+++
9.	Anthraquinones	+++	++	+	+	+++
10.	Glycosides	++	++	+	+	++
11.	Coumarins	++	+++	+	+	++
12.	Proteins	+++	++	+	+	+++

(-) Absent, (+) Present, (++) Moderate Amount, (+++) High Amount

Worldwide the herbal plants are majorly considered as the important origin of all raw material since the synthesis of various ancient and modern drugs [16]. The extensive research on therapeutic important plant species had played a vital role in the validation of such plants for treating various disorders, being a better developmental solution at low cost and with no side effects of such medication as a raw material [17-18]. Secondary metabolites had been reported having different therapeutic with biological utilities in medicinal uses. Because of high polarity solvents structures like methanol and ethanol it draws a heavy potential diverse plant constituents [19]. Phenolic compounds excessively found in these five medicinal plants are highly conducted for a huge range of various biological activities like antimicrobial, antioxidant, antifungal, anti-protozoal action, antiviral, antiallergic, antidiabetic, antispasmodic, antimutagenic, anti-inflammatory and

anticarcinogenic functions. Research had showed the phenolic compounds antimicrobial activity acts as bactericidal agents [20-21]. Plant saponins are also very important for treating of hypo and hyper cholesterols, hyperglycaemia, acts as anticancer agents, helps in weight loss, in food poisoning, acts as antifungal, antibacterial, antioxidant [21-22]. Flavonoids is a highly essential kind of polyphenols, it is group of organic heterocyclic compounds containing various important cellular and molecular properties as antimicrobial, antioxidant potential [22]. Due to these flavonoids, herbal plants possess a very high antioxidant action being water-soluble antioxidant, anticancer, antiviral agents with free radical scavenging activities [23]. All these three namely phenols, saponins, flavonoids were in these studied plants.

Alkaloids are a nitrogen based organic beneficial plant compound having antibiotic properties. It possesses analgesic, antidiabetic and antimicrobial properties [24]. Tannins contain anti-cancerous, anti-inflammatory, anti-ageing, anti-diuretic, antibacterial activities, anti-diarrhea, acts an astringent, used in soap, perfume industries [23-24], also have the ability for initiation of protein synthesis. Literatures had suggested these plants are tremendously rich in tannins compounds. Steroids are known as cardio-tonic, insecticidal, immunogenic, antimicrobial, helps in lowering high blood pressure and LDL cholesterol. Glycoside compounds plays a major part in anticoagulant, antitumor, anticancer, degranulating activities, inhibition of influenza virus [24-25]. Anthraquinones are highly responsible for coordination and regulation of body immunity, reported to had therapeutic roles in autoimmune diseases and diabetes. Terpenoids had anti-cancerous, antidiabetic, antimicrobial, antiviral, anti-inflammatory potentials, enhances insulin release through modifying glucose metabolism [26].

CONCLUSION

It is concluded that major, important origin of pigmented secondary metabolites i.e., pharmacologically active compounds including phenols, tannins, glycosides, flavonoids, steroid-sterols, carbohydrates etc are highly present in these selected medicinal herbs at a high (*A. somnifera*, *T. arjuna* and *A. indica*) and low (*B. monnieri* and *R. sceleratus*) amounts indicating better healing potentials. These are the phytochemicals that render various medicinal values of the such plants possessing numerous therapeutic properties. Hence, these five herbs are resulted in a range of huge therapeutically crucial plants. Further deep studies are necessary in evaluation, isolation, identification and understanding of its chemical characterization for further development of potent therapeutics against dysfunctional diseases.

Conflict of interests

Author has declared there is no competitive interest for this project work.

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