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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITIES OF IMPORTANT MEDICINAL PLANTS

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ABSTRACT

Medicinal plants are important sources for isolation of pharmaceutical drugs. The current available drugs are in many ways either inefficient or unaffordable to ever increasing forms of microbial infections. Screenings of medicinal plants used by the traditional medicinal healers are the main sources for formulation of herbal drugs. Phytochemicals present in plants are economically important sources of drugs, fragrances, pigments, food additives and pesticides. The present paper reports the analysis of qualitative, quantitative and antimicrobial properties of *Centella asiatica L.*, *Lawsonia inermis L.* and *Eclipta alba L. Centella asiatica* was found to have maximum contents of Flavonoids and phenolics whereas *Lawsonia inermis* has maximum contents of tannin. Antimicrobial analysis study showed that *Centella asiatica* has maximum antibacterial property against *Escherchia coli* MTCC68 and the plant extract of *Eclipta alba* showed maximum antimicrobial property against *Staphylococcus aureus* MTCC3160. The present work will help in understanding the comparative phyotochemical and antimicrobial properties of *Centella asiatica*, *Lawsonia enermis* and *Eclipta alba*. *Centella asiatica*, and set forward towards isolation and characterization of the bioactive compounds present in these medicinally important plants.

INTRODUCTION

The emergence of multidrug drug resistant microbes is of great concern to health management. This has rendered the current available antimicrobial agents insufficient to control microbial infections and create major public health problem (Cowan, 1999). Resistant to antibiotic due to extengens such as *Staphylococcus aureus* is of great concern (Cowan, 1999; Wattenberg *et al*., 1983). Rather than existing harmlessly in the gastrointestinal tract (GI tract), some strains of *E.coli* can disrupt body functions, resulting in diarrhoea. Other more pathogenic strains of *E.coli* can also affect the kidneys and nervous systems of victims, causing permanent damage and sometimes even resulting in death. Perhaps the most infamous strain is O157:H7, which was responsible for the Jack in the Box hamburger outbreak in 1993 and the more recent spinach outbreak in 2006 (Ingerson-Mahar and Ann 2011, 2011). The resistance of microbes to anti microbial agents can lead to treatment failure, morbidity and mortality of patients. Developments such as these have led to search for broad spectrum antibiotics, with less associated side effects. One of the best approaches to look for alternative effective drugs to counter the global health problem would be to screen for bioactive compounds present in medicinal plants reported in traditional practice, as it would be more cumbersome to analyse plants whose medicinal importance are not documented in any form. Plants are important for pharmacological research and drug development not only for using directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds, and also as pharmacological probes. The active principles of many drugs found in plants are secondary metabolites (Ghani, 1990). Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity viz. alkaloids, phenolics, essential oils and terpenes, sterols, flavonoids, lignins, tannins, etc. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides (Ramawat, 2007; Patwardhan *et al*., 2004; Ravishankar *et al*., 2007; Pal, 2007). Secondary metabolites also exert a profound physiological effect on mammalian systems; thus they are known as the active principle of plants. Most of the bioactive compounds identified from plants active against microorganisms are aromatic or saturated organic compounds, and are most often obtained initially through ethanol and water extraction (Lewis *et al*., 1995). *Centella asiatic* is a medicinal plant, native to most of the countries of Asia. The plant has been claimed to

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exert various physiological effects and is traditionally used for various ailments including wound healing, bronchitis, asthma, diabetes, kidney troubles, urethritis, liver complaints, allergy, cancer, diuretic, and hypertension and to improve mental ability (Goh, 1995). *Centella Asiatic* is also used as a potent drug in ulcer, depression and venomous insufficiency (Cho, 1981; Zheng and Qin, 2007). The plant is also found to improve the general behaviour and mental ability of retarded children (Appa, 1973). *Lawsonia inermis*, locally known as "Henna" has been found to exhibit Antibacterial, Antifungal and Dermatological properties. It is useful in colouring of skin, scalp and nails etc. Henna was also shown to have antidiarrhoel, diuretic, emmanagogue and abortifacient prophetically and found to be practically non-toxic (Lemordant and Foresteier, 1983).

In traditional medicine, henna plant is used to treat many diseases like oedema, bronchitis, menstrual disorder, rheumatism, haemorrhoids and even in jaundice, leprosy, pain, spleen enlargement, dysentery and skin problems (Rahmoun *et al*., 2010; Cuong *et al*., 2009; Bhuvaneshwari *et al*., 2002; Warrier *et al*., 1995). *Eclipta alba* is one of the important medicinal herbs with a role in the traditional medicine systems of the East. It is reported to possess antiseptic, analgesic, antipyretic, antispasmodic, antimicrobial and antiviral properties. *Eclipta alba* is reported to be effective for the retrieval of memory (Banji *et al*., 2007). It is hepatoprotective (Tabassum and Agrawal, 2004; Malhotra and Singh, 2007), anti-inflammatory (Arunachalam *et al*., 2009) and antimalarial (Bapna *et al*., 2007; Chenniappan and Kadarkarai, 2010). This plant is considered rejuvenative and good for hair, and a blackening dye for hair is obtained from this plant. The leaves of *Eclipta alba* are used against snake bites and scorpion stings. Thus, considering the broad uses of these plants, the present study was focus on comparing these three medicinal plants in terms of their major phytochemical constituents and their effectiveness against gram+ve and gram –ve bacteria.

MATERIALS AND METHODS

Collection of plant samples

The plants (Fig.1) were collected from the Khader area along the Yamuna, located at 28°59'N 77°01'E 28.98°N 77.02°E. *Eclipta alba L. and Lawsonia inermis L*. were collected from Village Hatkar, District Sonepat, Haryana, India during February 2011. *Centella asiatica L.* was collected from near the bank of the river Yamuna, Village Khatkar, District sonepat, Haryana, India during February 2011.

Fig. 1. Photograph of *Centella asiatica*, *Lawsonia enermis* and *Eclipta alba*

Processing of sample plants

The leaves of the plant samples were properly washed with tap water and rinsed with distilled water. The rinsed leaves were dried in an oven at a temperature of 35-40°C for 3 days. The dried leaves of each plant were pulverized, using a sterile electric blender, to obtain a powered form. The powdered forms of these plants were stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of aqueous extract of plant samples

5 g of the powdered plant leaves were taken in 50 ml distilled water. The suspension was gently rotated at 55°C for 6 hours, filtered with Whatman No.1 paper, and the filtrate was labeled as aqueous extract.

Preparation of solvent extracts of plant samples

5 g of the powdered leaves were taken in 50 ml methanol, and keep under gentle and continuous shaking on an orbital shaker (Stuart Scientific Orbital Shaker, UK) for 6 hours at 55°C. The suspension was then filtered using Whatman No. 1 paper to obtain crude methanolic extract. These compete steps were followed for all the three medicinal plants.

Quanlitative analysis of phytochemical constituents

Chemical analysis were performed on either the powdered sample, the aqueous extract (extracted liquid) or the secondary extract (100 mg extract powder dissolved in 1 ml of an appropriate solvent) using standard methods as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for steroids (Liebermann Burchard reaction)

300 µl of aqueous extract was added to 1 ml of chloroform and few drops of concentrated sulphuric acid was added along the sides of the test tubes. Reddish brown colour precipitate at the bottom of the test tubes indicated the presence of steroid.

Test for tannins

0.5 g of powdered sample was boiled in 20 ml of distilled water in a test tube and filtered using Whatman No.1. paper. 0.1% $FeCl_3$ was added to the filtered samples. The appearance of brownish green or a blue black colouration showed the presence of tannins.

Test for saponins (Froth Test)

300 µl of aqueous extract was added to 2 ml of distilled water in a test tube. The solution was vigorously shaken and observed for the stable froth persistence**.**

Terpenoids (Salkowski Test)

300 µl of aqueous extract was added to 1 ml of chloroform. Few drops of concentrated Sulphuric acid were carefully added along the sides of the test tubes. A reddish brown colour precipitate indicated the presence of terpenoid.

Test for cardiac glycosides (Keller Kiliani Test)

300 µl of aqueous extract was added to 1ml of Acetic acid followed by the addition of 300 µl of 10% Ferric Chloride and few drops of Concentrated Sulphuric acid along the sides of the test tubes. Brownish ring and green blue precipitates at the bottom of the test tube indicated the presence of Cardiac glycoside.

Test for quinines

2 ml of aqueous extract was added to 20 drops of Sodium hydroxide (NaOH). Appearance of blue green colour indicated the presence of quinones.

Tests for flavonoids

300 µl of aqueous extract was added to 1 ml of 10% ammonia and 1 ml of concentrated sulphuric acid. Disappearance of yellow colour indicated the presence of flavonoids.

Test for phenolic compounds

500 mg of powdered sample was dissolved in 5 ml of distilled water. Then few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Quantitative analysis on phytochemical constituents

Estimation of Phenolics

Isolation and estimation of phenolics was performed as suggested by Price *et al*. (1980). 5 g sample was homogenized in 200 ml acetone and kept overnight in shaker. Supernatant was collected and the residues were extracted twice with 10 ml acetone, filtered with Whatman No.1 paper and centrifuge at 3000x g for 10 min. The resultant Supernatant was used for estimation of phenolics. 1 ml distilled water was added to 25 µl supernatant. To this 25 µl acetone and 60 µl ferric ammonium sulphate was added, and kept the solution for 20 minutes at room temperature. Then 60µl potassium ferricyanide was added and kept for 20 minutes. The absorbance was measured at 720 nm using Quercetin as standard.

Estimation of Flavonoids

The isolation and estimation of flavonoids was carried out as per protocols suggested by Harborne (1975), and Lamaison and Carnat (1990). 5g of sample was acid hydrolyzed with 10 ml 1N sulphuric acid at 70^o C for 1 hour and neutralized with 0.5 ml of 10N sodium hydroxide. To this ethyl acetate was added and shaken well. Then the ethyl acetate layer was collected. This process of adding and collecting ethyl acetate was repeated twice. The ethyl acetate portions were and pooled together and evaporated to dryness. The residue was reconstituted with 1 ml methanol and assayed for total flavonoids content by measuring the absorbance at 430 nm.

Estimation of Tannins

0.5 g of plant sample was weighed into a 50 ml plastic bottle. 50 ml of distilled was added and stirred for 1 h. The sample was filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtered sample was then pipette out into test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)_6.3H_2O$. The absorbance is measured with a spectrophotometer at 395 nm wavelength within 10 min.

Preparation of plant extract for antibacterial screening

The plant materials were surface with 0.1% $HgCl_2$, and then properly wash with distilled water two to three times. The leaves of the plant were thoroughly washed and dried under shade at the room temperature. The dried materials were powdered in an electric grinder. 50 g of the dried powdered plant material was added to 200 ml of Methanol (w/v, 50g/ 200ml) , and kept under gentle and continuous shaking on an orbital shaker (Stuart Scientific Orbital Shaker, UK) for 6 hours at 55^oC. The suspension was filtered using Whatman No. 1 filter paper. Filtrate was evaporated at 60°C for 2 hours to obtain the extract powder. The secondary extract (100mg extract powder dissolved in 1 ml of Methanol) was used for analysis of antibacterial assay.

Screening of antibacterial activity

Antibacterial activities against the selected microorganisms were carried out using disc-diffusion method. A gram +ve bacterium *Staphyloccus aureus* MTCC3160 and a gram–ve bacterium *Escherichia coli* MTCC68 were obtained from Department of Microbiology, Ram Lal Anand College, University of Delhi, Delhi, India. The sterile Mueller Hinton agar media (pH 7.3) poured into sterile Petri dishes was spread

with bacterial cell cultures (100 µl of microbial cell suspension in 20 ml agar medium). Sterile Whatman No. 1 filter paper discs of 5 mm diameter and 0.2 mm thickness were impregnated with 20 µl of the secondary extract solution. These extract loaded paper discs were placed on the surface of the agar plates inoculated with bacteria. Then, the plates were incubated for (48h) at 37±1° C. 20 µg Vancomycin and Chloramphenicol antibiotic discs were used as positive controls. At the end of the incubation period (48 h) the antibacterial activity was evaluated by measuring the zone of inhibitions and comparing them with that of the standard references for 20 µl Vancomycin and Chloramphenicol.

Calculations

Activity index of the plant extracts were calculated by comparing the zone of inhibition of plant extract with that of vancomycin and chloramphenicol.

$$\text{Activity index} = \frac{\text{Zone of Inhibition of test sample}}{\text{Zone of Inhibition of the standard antibiotic}}$$

RESULTS

Quanlitative phytochemical analysis of the three medicinally important plants the presence of most of the tested phytochemicals in these plants. The results of the qualitative analysis are presented in Table 1.

Table 1. Phytochemical screening of leaf extracts of *Centettla asiatica, Lawsonia inermis* and *Eclipta alba*

Sl. No.	Compound	*Centella asiatica*	*Lawsonia inermis*	*Eclipta alba*
1.	Terpenoids	+ve	+ve	+ve
2.	Steroids	+ve	-ve	+ve
3.	Saponins	+ve	-ve	+ve
4.	Cardiac glycosides	+ve	+ve	+ve
5.	Alkaloids	+ve	+ve	+ve
6.	Phenolics	+ve	+ve	+ve
7.	Tannins	+ve	+ve	+ve
8.	Flavonoids	+ve	+ve	+ve
9.	Quinones	-ve	-ve	+ve

Positive = +ve, Negative = -ve

Quinones are absent in *Centella asiatica* and *Lawsonia inermis*. Quantitative estimation of phenoics, flavanoids and tannins were done by using Quercetin (at 720 nm for phenolics and 423 nm for flavanoids) and Gallic acids for preparation of standard curve as shown in table 2.The standard curves are shown in Figure 2, 3 and 4. The absorbance of *Centella asiatica, Lawsonia inermis and Eclipta alba* at 720 nm was 1.314 nm, 0.710 nm and 0.636 nm respectively, which in terms of concentration when derived from the standard curve for Phenolics turned out to be 602.5 µg/ml, 277 µg/ml and 250 µg/ml respectively. The absorbance of *Centella asiatica, Lawsonia inermis and Eclipta alba* at 430 nm was 1.701 nm, 0.739 nm and 0.587 nm respectively, which in terms of concentration when derived from the standard curve for Flavanoids turned out to be 972µg/ml, 600.1 µg/ml and 408.5 µg/ml respectively. Similarly, the absorbance of *Centella asiatica, Lawsonia inermis and Eclipta alba* at 393 nm was 0.718 nm, 1.064 nm and 0.816 nm, which in terms of concentration of Tannins turned out to be 653.5 µg/ml, 999 µg/ml and 741 µg/ml respectively. The results for quantitative analysis of these three major secondary metabolites showed that *Centella asiatica* has maximum contents of Flavonoids and phenolics. *Lawsonia inermis* showed maximum contents of tannin. Antibacterial activity of *Centella asiatica* and *Eclipta alba* showed that the plant extracts of *Centella asiatica* has maximum antibacterial property against *Escherchia coli* MTCC68 and the plant extract of *Eclipta alba* showed maximum Zone of Inhibition against *Staphylococcus aureus* MTCC3160, as provided in table 3 and 4.

Table 2. Absorbance of different concentrations (in µg/ml) of Quercetin, and Gallic acid

Concentration of Quercetin (µg/ml)	Absorbance (720nm)
100	0.271
200	0.509
300	0.814
400	0.929
500	1.286
600	1.392
700	1.576
800	1.869
900	1.921
1000	2.137

Concentration of Quercetin (µg/ml)	Absorbance (430nm)
100	0.087
200	0.164
300	0.373
400	0.552
500	0.692
600	0.738
700	0.976
800	1.321
900	1.681
1000	2.028

Concentration Of Gallic acid (µg/ml)	Absorbance (395nm)
100	0.159
200	0.329
300	0.426
400	0.581
500	0.629
600	0.673
700	0.738
800	0.882
900	0.992
1000	1.174

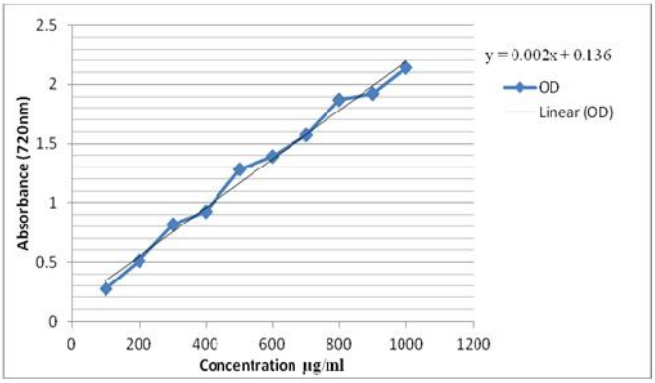

Fig. 2. Standard curve of Quercetin (at 720 nm) for quantitative estimation of phenolics

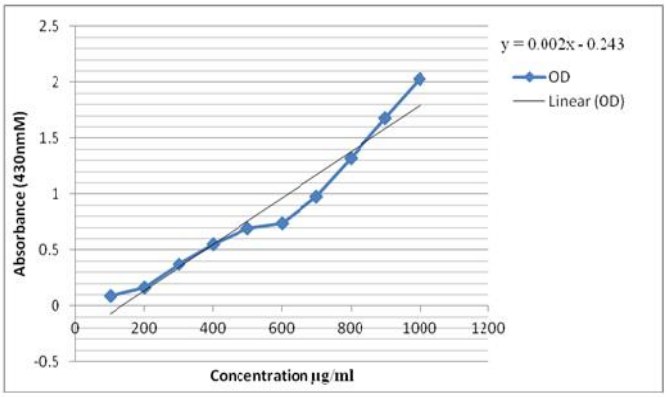

Fig. 3. Standard curve of Quercetin (at 430 nm) for quantitative estimation of flavonoids

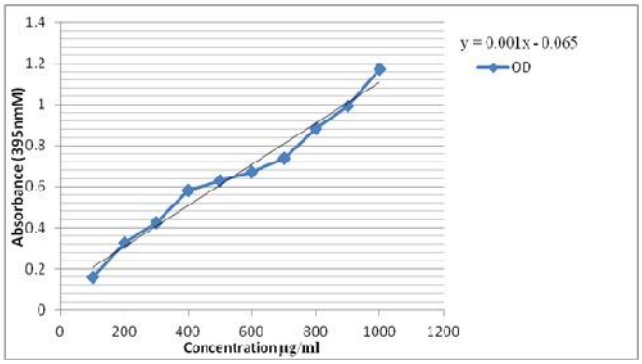

Fig. 4. Standard curve of Gallic acid (at 395 nm) for quantitative estimation of tannins

Table 3. Comparison of Zone of Inhibition (mm) on *Staphylococcus aureus* MTCC3160 and *E.coli* MTCC68 using different concentrations of secondary extracts of *Centella asiatica*

Sl. No.	Extract concentrations (µg/ml)	Zone of Inhibition (mm) on *Staphylococcus aureus* MTCC3160	Zone of Inhibition (mm) on *E.coli* MTCC68
1.	200	6.4	7.8
2.	400	8.1	8.2
3.	600	8.2	9.1
4.	800	8.3	9.2
5.	1000	8.6	9.5

Table 4. Comparison of Zone of Inhibition (mm) on *Staphylococcus aureus* MTCC3160 and *E.coli* MTCC68 using different concentrations of secondary extracts of *Eclipta alba*

Sl. No.	Extract concentrations (µg/ml)	Zone of Inhibition (mm) on *Staphylococcus aureus* MTCC3160	Zone of Inhibition (mm) on *E.coli* MTCC68
1.	200	7.9	5.8
2.	400	10.4	7.2
3.	600	12.6	8.1
4.	800	14.7	9.2
5.	1000	15.5	11.5

DISCUSSION

The present phytochemical analysis of *Centella asiatica* showed the presence of most of the major secondary metabolites, except Quinones which are the oxidation product of phenolics. Analysis on Tannins and Flavonoids showed that these compounds are present in *Centella acsitica* confirming the earlier analysis by Thangavel *et al*. (2011) and Mahfuzur *et al*. (2012) but in contrast to reports of Dharmendra *et al*., (2012) that reported Tannins and Flavonoids to be absent in *Centella asistica*. The presence of Saponins in this analysis also confirms that of Dharmendra *et al*. (2012) but it is in contrast to that of Thangavel., 2011 and Mahfuzur *et al*. (2012). Apart from these, all the results are in line with other earlier analysis. In *Lawsoniia inermis* Terpenoids like Steroids and Saponins, and Phenolics such as Quinones are absent in our analysis, however Wasim *et al*. (2013) reported steroidal compounds to be present. Iram *et al*. (2013) and Arun *et al*. (2010) reported the presence of Quinones which are in contrast to our present analysis. *Eclipta alba* was found to show positive result for all the tested compounds. Steroids were reported to be absent in aqueous extracts as reported by Manoj *et al*. (2012) but our report showed that steroids are also present in the aqueous extracts of this medicinal plant. The above mentioned variations in the observations of the photochemical analysis could be due to different collection time of the samples, as secondary metabolites are produced at specific stage condition during the life cycle of plants.

The accumulation of secondary metabolites in plants can even depend on stages of growth of the plant. In most of plants, maximum accumulation of metabolites occurs during the time of flowering, and lesser accumulation occurs at the fruiting stage. Thus, the time of harvest or field collection of the samples can influence the quality and quantity of the secondary metabolites. Other factors that can affect the qualities of phytochemical compounds present in plants includes factors such as soil, light, water, temperature and nutrient condition provided to the plant. The methods used in collection of samples from the wild, method of cultivation, harvest, post-harvest processing, shipping, and storage can also influence the physical appearance as well as the phytochemical constituents of the plant samples. Further, contamination by microbial and chemical agents (pesticides, herbicides, heavy metals), as well as infection by pest and pathogens can alter the chemical constituents of the plants. Qualitative analysis of three important secondary metabolites viz. Phenolics, Flavonoids and Tannins showed that *Centella asiatica* has maximum contents of Flavonoids and Phenolics. *Lawsonia inermis* showed maximum contents of Tannin. These results are in favour of the various reports on the broader and more effective used of *Centella asiatica* in various health problems, the property of which are depicted here in the present qualitative analysis.

The higher Tannins concentration of *Lawsonia inermis* from the present analysis confirms its highly known use in colouring of skin, hair and its antimicrobial properties. The antimicrobial assay showed *Centella asiatica* to be more effective against Gram +ve bacterium *Escherchia coli* MTCC68, and the extracts of *Eclipta alba* showed maximum Zone of Inhibition against the Gram –ve bacterium *Staphylococcus aureus* MTCC 3160. These observations validate the used of these plants for antimicrobial activity. Plants produce three major groups of secondary metabolites: the terpenoids, the alkaloids, and the phenylpropanoids and allied phenolic compounds. The first

pure compound to have been discovered in any series of chemically or developmentally related therapeutic agents is called as the drug prototype (Walter, 2005). In some cases the prototypes continue to serve as medicinal compounds in their own right, but in other new more effective analogues can be derived from the prototype, rendering the prototype obsolete. One-fourth of the drugs approved during the period 1981–2002 was either secondary metabolites (natural products) or based on natural products (Newmann, 2003). WHO estimated that up to 80 % of the population in Africa and the majority of the populations in Asia and Latin America still use traditional medicine for their primary healthcare needs (Anon, 2003). The Phenolics such as Flavonoids can influence the radical scavenging activity of cells, and also has inhibiting activity against hydrolytic and oxidative enzymes, and can act as anti-inflammatory agent (Frankel, 1995). Flavonoids may help in providing protection against some diseases such as oxidative stress and cellular damage (Burlon and Ingoid, 1984).The mechanism of action of flavonoids is through scavenging or chelating process (Cook and Samman, 1996; Kessler *et al*., 2005).

It has been recognized for some time that several classes of flavonoids play a significant role in many physiological processes and show antioxidant and fungicidal activity (Larson, 1988) and are natural antihistamines. A variety of modifications of the flavonoid skeleton lead to a large class of compounds that includes isoflavones, isoflavonones and chalcones. Some isoflavones are now been marketed as therapeutic agents for menstrual disorders. Tannins are other complex types of phenolics which can bind to proteins and carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibition of microbial growth. They have been used since past as tanning agents and they posses astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial activities (Killedar, 2010).They also prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sadipo *et al*., 1991). Increasing attention is also being paid to the use of tannins as antimicrobial agents (e.g. wood preservation) or prevention of dental caries. They impart flavour to wines. Recently, evidence has been obtained in support of their potential value as cytotoxic or antineoplastic agents. In addition, tannins are now being used in the manufacture of plastics, paints, ceramics and water softening agents.

Quinone mediated ROS can cause cellular damage through alkylation reactions with lipids, proteins, and DNA (Bolton *et al*., 2000). Depending on the particular system, quinones can act as antioxidants and protect healthy cells against ROS, or act as cytotoxic agents, generating ROS in unhealthy cancer cells. Quinones can target the mitochondria and re-establish electron transfer in deficiency states. For example, in antiphospholipid syndrome, treatment with co-enzyme Q has been shown to alter mitochondrial dynamics resulting in lower oxidative stress and slowing of the accelerated atherosclerosis (Perez-Sanchez *et al*., 2012). Co-enzyme Q has also been shown to prevent retinal cell apoptosis when given as eye drops in mouse models of kainate-induced retinal damage (Lulli *et al*., 2012). Quinones are also being investigated for the treatment of mitochondrial diseases (Enns *et al*., 2012) as well as age-related diseases (Skulachev *et al*., 2009). Alkaloids were known to humans for several centuries. They are a diverse group of low-molecular-weight, nitrogen-containing compounds found in about 20% of plant species. Alkaloids are one of the largest groups of phytochemicals that have led to the invention of powerful pain killer medications (Kam and Liew, 2002). Alkaloids are used in various therapeutic purposes such as analgesics (morphine and codeine), anticancer agent (vincristine and vinblastine), gout suppressant (colchicines), muscle relaxant (+) (tubocurarine), antiarrythemic (ajmalicine), antibiotic (sanguinarine), antimalarial agent (quinine), sedative (scopolamine), and even in beverages (caffeine in tea and coffee) Ramawat (2007). Terpenoids are derived from the basic five carbon isopentane unit and the number of these units in the molecule determines its classification. Terpenoids are located in the vacuole of the leaf or root of plants while some are located outside the leaf (Harborne, 2003). Some terpenoids actually exist as glycosides, whilst others contain nitrogen and are therefore known as terpene alkaloids (Goodwin and Mercer, 1975). Terpenoid indole alkaloids comprise a group of about 3000 compounds with well-known compounds such as antineoplastic agents' vinblastine and camptothecin, the antimalarial drug quinine and the rat poison strychinine. Steroids are modified triterpenes and have profound importance as hormones (androgens such as testosterone and estrogens such as progesterone), coenzymes and provitamins in animals.

Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics (Callow, 1936). Steroids have been reported to have antibacterial properties. It has been reported to affect major detoxifying enzymes (Raquel, 2007). Triterpenoids produce several pharmacologically active groups such as steroids, saponins and cardiac glycosides. Saponins which act as bioactive antibacterial agents in plants are also used to treat hypercholesterolemia, hyperglycemia and obesity. The presences of cardiac glycosides are known to play a major role in heart muscles by inhibiting Na+ and K+ pump that increase the availability of sodium ions and calcium ions to heart muscles which improves cardiac output and reduce heart distension. Thus, they are used in the treatment of congestive heart failure and cardiac arrhythmia (Schneider and Wolfling, 2004). The presence of tested phytochemicals in the plants helps in setting future approach to isolation of these compounds in pure forms that can act as a direct source of drugs or as prototypes from which more beneficial compounds can be derived. In addition to their direct use as drugs or drug prototypes the secondary metabolites of plant origin, such as phorbol esters and genistein, can be used as "pharmacological probes that help to understand the mechanism of action of intracellular signal transductions and biological mechanisms related to human disease.

Conclusion

Despite the common notion that phytochemical compounds are safe, they all have inherent risks just like any synthetic drugs.

Thus, it is important to evaluate and understand the side-effects, appropriate doses, so as to avoid unwanted health effects from using these medicinal plants. Inappropriate use of the plants also needs to be regulated to avoid over exploitation and to ensure their availability for the future. Continued phytochemical screening, analysis and isolation of antimicrobial drugs from these and other potential medicinal plants sources need to be encourage, without limiting only to screening bioactive compounds, but areas in these research should be pushed forward towards isolation of the pure compounds responsible for the observed medicinal properties so as to able to discover the most effective drugs to counter the ever increasing multidrug resistance properties of the microbes.

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