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7.7. SIGNIFICANT FINDINGS
7.1. INTRODUCTION

Throughout the ages, humans have relied on natural world for their essential needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours, fragrances as well as medicines (Cragg and Newmann, 2005). The nature has provided the storehouse of remedies to cure all ailments of mankind. The traditional herbal medicines are still practised in large part of our country mostly in tribal and rural areas. In many developing countries, a large section of population relies on conventional practitioners, who are dependent on herbal folk medicines for their most important health care. Since the use of these herbal folk medicines is increasing, the issues of safety, quality and efficacy in industrialized and developing countries have cropped up. Thus, plants have formed the basis of complicated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Even though some of the therapeutic properties credited to plants have proven to be incorrect, but, as a matter of fact, medicinal plant therapy is based on the findings of hundreds and probably thousands of years of use.

Environmental disasters are caused by human activity (Diamond, 2005). It can also affect agriculture, biodiversity, the economy and human health. The causes of disasters include pollution, depletion of natural resources, and scarcity of pure water and occurrence of various infectious diseases. Out of the other infectious diseases, the most prevalent is malaria disease which is mosquito-borne infectious disease of humans and other animals caused by eukaryotic protists of the genus Plasmodium.

Malaria is a foremost parasitic disease in the world, mainly in India. It is generally ubiquitous in the midst of insect-borne diseases. It is conscientious for 500 million new cases and 2 to 3 millions deaths every year on the whole amongst children under five years as well as pregnant women. It is a traditional example of a disease that affects the efficiency of individuals, families as well as the entire society. It is widespread in the poorer, moreover, less-developed countries of the world (Ekthawatchai et al., 1999). The erstwhile hard strike tropical areas comprise East Asia, China with India. It was estimated that about 40% of India’s malaria cases is caused by Plasmodium falciparum. This disease results from the multiplication of Plasmodium parasites within red blood cells, which cause symptoms that include fever as well as headache and in several cases progressing to coma or death.
*P. falciparum* is the generally well-known etiological agent intended for human malaria and has turned out to be gradually more opposed to standard antimalarial medications. The initial antimalarial drug is quinine which was isolated from the bark of *Cinchona* species (Rubiaceae) in the year 1820. It is one of the oldest and the most important antimalarial drug which is still dominantly used today (Beckmann, 1958). In 1940, an additional antimalarial drug chloroquine was synthesized. Until recently this was one of the significant drugs used for the treatment of malaria (Bharel *et al*., 1996).

Medicinal plants and derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals (Vanwyk and Wink, 2004). In the previous few decades there has been an exponential increase in the field of herbal medicine. It is getting popularized in developing as well as developed countries due to its natural origin in addition to lesser side effects (Patel, 2001). At the current juncture, the modern conventional healthcare is loaded with great problems of unsafe medicines, chronic diseases, resistant infections, auto-immune disorders as well as degenerative disorders of ageing, despite great scientific advances. India possesses almost 8% of the approximate biodiversity of the world with just about 0.126% million species (Jain *et al*., 2006). Currently, Ayurveda coexists with modern system of medicine, and is still broadly used and practised. About 30% of the currently used therapeutics is of natural origin.

India has about 2000 species of medicinal plants and a vast geographical area with high production potential and varied agro-climatic conditions. For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of plant compounds for pharmaceutical purpose is steadily increasing. In current years, the secondary plant metabolites (phytochemicals) with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Standardization of Ayurvedic or Siddha formulations is the prime need of the current time. Many of them do not have uniform standards and analytical procedures to justify their quality and purity. Modern techniques such as HPTLC, HPLC, TLC etc. can be used to develop the methods for the quantification of marker compounds.
in these types of multi component herbal formulations (Jeganathan and Kannan, 2008).

Oxygen is essential for the survival of all on this earth. Though oxygen is important for life, overload oxygen can have harmful effects. When oxygen is metabolised by the body, it creates substances called free radicals and this causes damage to our cells and then the antioxidants, natural nutrients in food, help in destroying these free radicals and minimise damage to our cells (Omenn et al., 1996). Some examples of antioxidants are beta-carotene, lycopene, vitamins C, E, and A and other substances. It can be classified into two classes based on their sources i.e. natural or synthetic antioxidants. Natural antioxidants are extracted from plant and animal sources. Synthetic antioxidants are prepared synthetically in the laboratory. Since ancient times, plants have been an excellent source of medicine. Ayurveda and other Indian literature reveal the use of plants in treatment of various human ailments. India has about 45,000 plant species and among them, some thousands have been claimed to have medicinal properties. The plants mentioned in ancient literature are used traditionally for various infectious diseases which show antioxidant property. The present study reviews 20 such plants which have been used in the Indian traditional system of medicine and have shown experimental antioxidant activity. Indian plants which are most effective and the most commonly studied in relation to various infectious diseases and their complications are: *E. campestris* (Salamgatta), *P. retrofractum* (Chvya), *P. nubicola* (Nirvanshi), *V. wallichii* (Tagar), *Z. sativa* (Unnab), *R. hypocretreiformis* (Phang), *B. retusa* (Ras-Kasay), *W. fruticosa* (Fire Flame Bush), *S. surattense* (Bhat-Kateli), *C. intybus* (Kasani), *M. sylvestris* (High-Mallow), *O. bracteatum* (Sedge), *C. colocynthis* (Bitter Cucumber), *D. inoxia* (Datura), *R. communis* (Castor), *Q. infectoria* (Majuphal), *L. usitatissimum* (Linseed), *S. nigrum* (Makoy), *C. longa* (Turmeric) and *A. calamus* (Vasa).
7.2. REVIEW OF LITERATURE

The use of plants as source of therapeutic drug for the treatment of many diseases is as old as human civilization. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal therapeutic drugs proved successful (Sofowora, 1982). Plants usually create numerous secondary metabolites which represent an imperative source of microbicides, pesticides in addition to many pharmaceutical drugs. Plant foodstuffs still stand as the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe et al., 1998).

The advantageous therapeutic effects of plant materials characteristically result from the combination of secondary products present in the plant. The therapeutic actions of plants are exceptional to particular plant species or groups and are dependable with this concept as the combination of secondary products in a particular plant which is taxonomically distinct (Wink, 1999).

At present days, medicinal plants receive attention to research centres because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan et al., 2009; Lozoya and Lozoya, 1989). These are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Plant based natural constituent can be derivative from some part of the plant in the vein of bark, leaves, flowers, roots, fruits, seeds etc (Gordon and David, 2001).

The Phenylpropanoids (PPs) which belong to the major assembly of secondary metabolites formed by plants are mostly in respond to biotic and abiotic stresses. The metabolic pathways of these Phenylpropanoids biosynthesis in plants and the molecular basis in favour of the defensive act of phenylpropanoids in plants is their antioxidant and free radical scavenging properties. The natural as well as synthetic phenylpropanoids are used as antioxidants for medicinal purposes. The plants, free radical-driven, molecular and cellular processes modulated by phenylpropanoids in...
human cell cultures *in vitro* and in the *in vivo* animal models of tumors, inflammation, and cellular damage are furthermore reported. (Korkina, 2007).

The medicinal plants which are used generally having methanolic crude extracts were screened in support of their free radical scavenging property using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and ascorbic acid as standard antioxidant. It was found that on the whole antioxidant activity of green tea (*Camellia sinensis* Linn.) be the strongest out of all the methanolic extracts which exhibit antioxidant activity appreciably. The methanolic extracts having IC50 range between 6.7 ± 0.1 and 681.5 ± 8.4 μg/ml and so as to of ascorbic acid was 8.9 ± 0.1 μg/ml. It was concluded that the utilization of these type of spices would apply numerous advantageous effects by asset of their antioxidant activity (Nooman *et al.*, 2008).

Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavour and cosmetic industries. Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper prices than modern medicine (Mann *et al.*, 2008).

### 7.3. OBJECTIVE

- Identification of plants with ethno-medicinal background as potential Antimalarial agents/ Antioxidant agents.
- Photochemical characterization of active extracts for scientific co-relation of bioactive and its antioxidant potential.
- TLC for characterization of active extracts.
- HPLC (qualitative and quantitative studies).
- Antioxidant efficacy: Extraction and isolation of active extract/fraction/bioactive as antioxidant agents by established protocols of Takao *et al.*, 1994 using DPPH as radial scavenging agent which entrap the free radicals to work as potentials *in vitro* experimentations.
7.4. MATERIALS AND METHODS

7.4.1. Collection

Authentic samples: Various market samples of 20 medicinal plants were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

7.4.2. Identification

All the samples were authenticated and each was given an identification number. The identification was as follows:

These samples were authenticated and submitted in Ethno-medicinal Herbarium, Centre of Excellence (funded by DST), MGiaS, Jaipur (Rajasthan).

7.4.3. Processing of plant materials

During the course of the study, each sample was screened for its foreign matter and milled before use.

7.4.4. Experimental details

Various Tests were performed on 20 medicinal plants for the following studies-

1. Extraction
2. Phytochemical Screening.
3. TLC (Thin Layer Chromatography) and High pressure liquid chromatography (HPLC).
4. Antioxidant Potentials of Methanolic extract of plant.
5. NMR spectrum of isolated pure compounds
6. Statistical analysis

7.4.4.1. Extraction

For phytochemical profile of selected species each of the dried and powdered (gm.) test samples were soxhelt extracted in Methanol for 6 hours. The extract was filtered, evaporated to dryness and weighed. This extract was used for phytochemical screening of plant active compound.
7.4.4.2. Phytochemical Screening

Phytochemical screening was performed using standard procedure:

1. Test for reducing sugars (Fehlings test)
2. Test for terpenoids (Salkowski test)
3. Test for flavonoids
4. Test for tannins
5. Test for saponins
6. Test for alkaloids

7.4.4.3. TLC (Thin Layer Chromatography) and High pressure liquid chromatography (HPLC)

Concentrated extracts were used for isolation and characterization of compounds. Crystallization by the solvent Hexane:Acetone (3:1) was performed to separate pure bioactives which was checked by thin layer chromatography (Harborne, 1973). Also, various Indian medicinal plants were subjected onto the HPLC analysis using Shimadzu Model LC2010 AHT Auto Sampler (UV-VIS Detector).

7.4.4.4. Antioxidant Activity

In present investigations attempts were made to screen selected 20 Indian medicinal plants as potent antioxidant agents to cure the future generation from junk food generated degenerative diseases. Further, these extractives can also be used as herbal drink. Screening of various extracts was performed against DPPH (1, 1'-diphenyl-2-picrylhydrazl) assay (Takao et al., 1994) for antioxidant activity (qualitative and quantitative estimation) and thus, these potentials can be used not only as antioxidant agents but also as safe herbal drinks.

7.4.4.5. NMR spectrum of isolated pure compounds

The compound was subjected to NMR analysis (model Brukur-DPX-300 MHz, using CDCL$_3$ and DMSO- d$_6$ as an internal reference) along with the standard reference compound.

7.4.4.6. Statistical analysis

All data were analysed by standard statistical analysis methods. The results were compared with standard data available on websites.
7.5. RESULTS AND DISCUSSION

7.5.1. Phytochemical screening

In present study 20 medicinal plants have been phytochemically evaluated for various tests i.e. reducing sugar, saponins, tannins, flavonoids, terpenoids and alkaloids.

Reducing sugar was present in *P. retrofractum*, *Z. sativa* and *C. longa*. Saponins was present in *R. hypocrateriformis*, *W. fruticosa*, *S. surattense*, *M. sylvestris*, *O. bracteatum*, *R. communis* and *A. calamus*. Tannins was present in *B. retusa*, *W. fruticosa*, *S. surattense*, *C. intybus*, *O. bracteatum*, *D. inoxia* and *Q. infectoria*. Terpenoids was present in *P. retrofractum*, *D. inoxia*, *Q. infectoria*, *L. usitatissium* and *S. nigrum*. Flavonoids was present in *P. retrofractum*, *R. hypocrateriformis*, *C. intybus*, *M. sylvestris*, *C. colocynthis*, *Q. infectoria*, *C. longa* and *A. calamus*. Alkaloids was present in *E. campestris*, *Piper retrofractum*, *P. nubicola*, *V. wallichi*, *M. sylvestris*, *O. bracteatum*, *C. colocynthis*, *D. inoxia* and *C. longa*.

7.5.2. Thin Layer Chromatography (TLC) And High pressure liquid chromatography (HPLC)

TLC has been regarded as a simple, rapid and inexpensive method for the separation, identification and semi-quantification of a wide variety of substances by scanning chromo-strips with or without detecting reagents, under normal or UV light. The resultant differential chromatographic “fingerprints” can actually be used as “markers” for each extract in a particular solvent system separating the compounds at specific R\(_f\) value, which will differ to other plant extracts. These R\(_f\) values are simple, reproducible and thus reliable marker to verify the purity of the crude drugs. In view of this, TLC investigations of different plants were carried out. TLC fingerprints were generated from petroleum ether extracts of *E. campestris*, *P. retrofractum*, *P. nubicola*, *V. wallichi*, *Z. sativa*, *S. nigrum* and *S. surattense* using solvent system Hexane: Acetone (3:1). By these fingerprints, the quality control of an authentic drug in various quarantines can be achieved.

HPLC is a rapid, reliable as well as data-oriented method which is used for quality control of a variety of drugs and provides enough characteristics that allow
these to be distinguished. Formerly, various TLC procedures were worked out for diverse drugs using petroleum ether solvent. These systems had the boundaries of resolution, sensitivity in addition to implementation for quantification; on the contrary HPLC has been the technique of choice for the division and quantification of natural products as isocratic separations are favoured, anywhere possible. Besides, they do not require complex gradient systems, and thus, can be easily reproduced and eradicate the necessity of re-equilibrating the column between the runs. In the present study, HPLC was performed for Lupeol, β-Sitosterol, Stigmasterol and Lupeol run in methanol under 254 nm, the rt time recorded at 17.656, 18.138 and 6.714 which showed that as the column size increases it affects the retention time (column size α rt). It also affects the peak sharpness.

HPLC profile of petroleum ether extract of _S. surattense_ have characteristic peaks at retention time 2.880, 3.185, 3.342, 3.478, 4.085, 6.714 (Stigmasterol), 10.003, 17.687 (Lupeol), 18.154 (β-Sitosterol) whereas _E. campestris_ have peaks at retention time 2.885, 3.193, 3.321, 3.493, 4.075, 18.138(β-Sitosterol). These peaks showed that there are different compounds and characteristic fingerprints for each drug to judge in an herbal formulation. There normalized fingerprints are principal markers that can check the purity/impurity of drug at very low concentration.

7.5.3. Antioxidant Activity

An enzyme or other organic molecules that can neutralize the harmful effects of oxygen in tissues known as antioxidants. The major characteristic of an antioxidant is its capacity to “trap free radicals”. In nature, human body produces chemicals known as ‘free radicals’ which cause irreversible damage (oxidation) of nucleic acids, proteins etc. These can leave the body exposed to advanced ageing, cancer and cardiovascular as well as degenerative disease like arthritis. Although, not only body has a natural antioxidant mechanism that protects from most cells damage, many plants and their products which are part of our diet also exhibit antioxidant potential.

Primary source of naturally occurring antioxidants are whole grain, fruit, vegetables etc. which can decrease the degenerative diseases. Use of DPPH for quick, simple and inexpensive measurement of antioxidant capacity of food involves to trap the free radicals as well as to quantify the antioxidants in complex biological system.
This way of quantification of genuine antioxidants can be used as marker for standardization. Each plant has its own antioxidant potential and ED$_{50}$ value. With simple analytical techniques, such markers can be generated in short time economically and effectively. In present study, attempts have been made to quantify the antioxidant potentials of methanolic extracts of various medicinal plants using DPPH assay method and recording their absorbance at 517 nm.

**Qualitative assay:** The qualitative antioxidant assay using DPPH spray on TLC plates bearing spots of petroleum ether extract of genuine and adulterants of various plants indicated the presence of antioxidant components in a variable manner, in *E. campestris*, *P. retrofractum*, *P. nubicola*, *V. wallichii*, *Z. sativa*, *S. nigrum* and *S. surattense*.

**Quantitative assay:** The authentic sample of 20 medicinal plants showed some antioxidant properties in a quantitative DPPH assay. The ED$_{50}$ of ascorbic acid comes out to be 0.646 μg/ml where as *E. campestris* (ED$_{50}$= 1.593 μg/ml) showed high antioxidative ED$_{50}$ value as compared to others. While others showing, *P. retrofractum* (ED$_{50}$=1.449 μg/ml), *P. nubicola* (ED$_{50}$=1.002 μg/ml), *V. wallichii* (ED$_{50}$=1.556 μg/ml), *Z. sativa* (ED$_{50}$=1.409 μg/ml), *R. hypocreareiformis* (ED$_{50}$=0.995 μg/ml), *B. retusa* (ED$_{50}$=0.277 μg/ml), *W. fruticosa* (ED$_{50}$=0.658 μg/ml), *S. surattense* (ED$_{50}$=0.517 μg/ml), *C. intybus* (ED$_{50}$=0.409 μg/ml), *M. sylvestris* (ED$_{50}$=0.234 μg/ml), *O. bracteatum* (ED$_{50}$=0.479 μg/ml), *C. colocynthis* (ED$_{50}$=1.311 μg/ml), *D. inoxia* (ED$_{50}$=0.719 μg/ml), *R. communis* (ED$_{50}$=0.838 μg/ml), *Q. infectoria* (ED$_{50}$=0.254 μg/ml), *L. usitatissimum* (ED$_{50}$= 0.710 μg/ml), *S. nigrum* (ED$_{50}$= 0.490 μg/ml), *C. longa* (ED$_{50}$=0.992 μg/ml), *A. calamus* (ED$_{50}$=0.763 μg/ml).

**7.5.4. Isolation of Bioactive Compounds**

The NMR spectroscopy and its spectral pattern are highly sensitive and have the capacity to differentiate not only the compound but also the extracts of different plant materials of common identity like genuine and sample.

In brief, thus, a “Monograph” may be concluded from the significant findings of the present study. Besides this, the resulting conclusions are of applied nature. Earlier, phytochemical analysis were in use to check the sample but, from the present findings newer techniques such as TLC fingerprints and antioxidant techniques have
been generated for their use to separate out the potential ability of a drug to work against malaria and thus, provide a tool for curing the disease in which RBC level falls increasingly. Besides, it provides an antioxidant agent which can be used as an anti-malarial herbal nutraceutical source for therapeutics. It is noteworthy that some of these new biological efficacies (antioxidant) will further enhance the shelf life, drug potentials, and effectivity of the herbals. In present research, attempts were made to isolate various pure bioactives from various Indian medicinal plants and it is remarkable that Lupeol, β-Sitosterol and Stigmasterol were isolated which have potential source as antioxidant agents. Thus, these plants have a great role to play as drugs or therapeutic targets, in future.

7.6. CONCLUSION

Plants are the essential chemical factories having various therapeutic potentials. Due to Environmental disasters, various diseases are on rise. The nature has provided the storehouse of remedies to cure all ailments of mankind. Thus, plants have formed the basis of complicated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Even though, some of the therapeutic properties credited to plants have proven to be incorrect, still the medicinal plant therapy is in existence for thousands of years. In recent years, the threat of bio-piracy of herbals has resulted into degradation of quality. As a result, most herbs are likely to be vanished causing possible risks of human life.

The Indian medicinal plants are in great demand as they are known for their medicinal properties and newer compounds formulations are continuously introduced in the market. In present investigations, plants with ethno-medical background and therapeutic potential were screened using traditional and spectroscopic analysis for their various antagonistic properties, like antioxidant properties. In present study, we have found that most of the biologically active phytochemicals are present in the extracts of the plants investigated.
7.7. SIGNIFICANT FINDINGS

Fast rising of multi-drug resistant malaria parasite has necessitated the development of new-fangled chemotherapeutic agents to conflict malaria. Medicinal plants are damaged in numerous countries to handle malaria disease and are considered to be not as much of toxic as allopathic hypoglycemic drugs whereas herbal medicines make available realistic means for the curing of malarial diseases. This study aims to explore for novel anti-malarial drugs.

The present study indicates that the flavonoids are present in P. retrofractum, C. intybus, R. hypocratiformis, M. sylvestris, C. colocynthis, Q. infectoria, C. longa and A. calamus. The occurrence of flavonoids in huge quantity is rationally proportional to the antioxidant activity. Hence, it is evident that occurrence of flavonoids will improve the antioxidant activity and promote a drug for treatment of malaria. Besides, further work on isolation of the bioactives might lead to the production of antimalarial drugs from medicinal plants. All the plants exhibited strong antioxidant activity more or less. The occurrence of flavonoids in plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids are the phenolic compounds and plant phenolics are a major group of compounds that perform as primary antioxidants or free radical scavengers.

TLC investigations of various Indian medicinal plants were carried out. TLC fingerprints were generated from petroleum ether extracts of E. campestris, P. retrofractum, P. nubicola, V. wallichi, Z. sativa, S. nigrum and S. Surattense. When Petroleum ether extracts of various plants run in solvent system Acetone: Hexane (1:3) with standard i.e. β- Sitosterol, Stigmasterol, Lupeol , they show the presence of various standard compounds at different Rf value. The best result was shown in S. surattense which shows the presence of β- Sitosterol at Rf -.60 (Pinkish Purple), Stigmasterol at Rf -.63 (Purple) and Lupeol at Rf -.72 (Pink). By these fingerprints, the quality control of an authentic drug can be achieved.

Extracts of various medicinal plants were subjected to viewing for their probable antioxidant activity. The two corresponding test systems, specifically DPPH free radical scavenging along with reducing power, were used for the chemical analysis. DPPH is a stable free radical with a distinguishing absorption at 517 nm. It
was used to swot the radical scavenging effects of extracts. As antioxidants contribute protons to these radicals, the absorbance decreases. The decrease in absorbance is engaged as an evaluator of the extent of radical scavenging. Free radical scavenging capacity of the extracts as well as standard (Ascorbic Acid), measured by DPPH assay was observed. The authentic sample of 20 Indian medicinal plants showed some antioxidant properties in a quantitative DPPH assay. The ED$_{50}$ (Effective dose) of ascorbic acid comes out to be 0.646 μg/ml where as $E$. $campestris$ (ED$_{50}$= 1.593μg/ml) showed high antioxidative ED$_{50}$ value as compared to others. Consequently, this research suggests that these plants have inherent antioxidant activities which can counteract the oxidative damage induced by the malaria parasite. This may be one of their modes of action in malaria therapy.

The consequence of herbs in the management of human ailments cannot be over emphasized. It is apparent that the plant kingdom harbour an inexhaustible resource of active ingredients very useful in the management of many intractable diseases. In addition, the active components of herbal remedies have the benefit of being combined with many other substances that emerge to be inactive. Though, these corresponding components give the plants as a whole safety and effectiveness much superior to that of its isolated and pure active components. Oxidative stress plays one of the major roles for growth of anaemia in malaria. The junk food as well as environment of now a days lead to degenerative diseases to mankind. These degenerative diseases will provide free radicals and it will be the major origin of degenerative diseases including malaria, whereas a medicinal plant represents a rich source of antioxidant agents. Plants are used medicinally in different part of countries and are a rich source of many potent and powerful drugs. The use of medicinal plants to treat human disease has its ancestry in ancient times. A broad range of medicinal plant part is used for extract as raw drugs as well as they possess various medicinal properties.