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Reviews on Quantitatively Analysis of Phytochemical in Traditional Medicinal Plants

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Abstract

Most medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. This paper explains about the analysis of Phytochemicals of medicinal plans by HPTLC-MS is more advantages than other methods and reviews on the comparing the results of different methods with the results of HPTLC-MS. In addition to this, emphasize on the previous method is to be more sensitive, low cost and that detect even at low concentration than other technique. There are several methods involved in the analysis of medicinal plants such as spectrophotometry, spectrofluorimetry or electro-analysis, HPLC, LTC as well as HPTLC. From those, to our knowledge, the HPTLC is most important methods to analysis the medicinal plants. The proposed HPTLC-MS method presents high selectivity and sensitivity, adequate accuracy, reliable, reproducible and low-cost.

Key words: Medicinal Plants; HP TL C-MS; Phytochemicals; Bioactive compounds

Introduction

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [1]. Medicinal plants are best owed with large number of pharmaceutically useful compounds which can be studied for investigation of new drugs for many serious diseases like cancer, tumours, AIDS and many human degenerative diseases. Medicinal plants are known to produce certain bioactive molecules which inhibit bacterial or fungal growth [2]. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [3]. Medicinal plants act as an indigenous source of new compounds possessing therapeutic value and can also be used in drug development. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO [4]. Because of the growing recognition of natural products the demand for medicinal plants has been increasing all over the world.

They have minimal toxicity, are cost effective and pharmacologically active, and provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects [5]. Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites [6]. Several methods mainly based on spectrofotometry, spectrofluorimetry or electro-analysis was used for phytochemical analysis of medicinal plants [7-9]. This reviews paper reviled that more sophisticated analytical methods have been used for analysis of phytochemical of medicinal plants including High-Performance Tine layer Liquid Chromatography (HPTLC) coupled with Mass-Spectroscopy (MS) rather than those of spectrofotometry, spectrofluorimetry or electro-analysis because the results of those methods have some interferences [10-12].

High-Performance Tine layer Liquid Chromatography (HPLC) coupled with Mass-Spectroscopy (MS) system can be used for determination of different components of medicinal plants in reliable and reproducible methods of analysis that provides essential information regarding the compositional quality of an herbal substances. Some advantages of this technique over the others, low cost and a relatively simple test methods. It does not require advanced sample preparation methods or high levels of expertise. Samples amounts are relatively small and it is a more sensitive technique compare with HPLC and other techniques as well as suited to detecting contaminants.

Plant material

Different medicinal plant samples such as Ranunculus arvensis, Equisetum ravens, Carathamus lanatus and Fagonia critica were collected from different areas. The plant samples were rinsed with tap water and then with de- ionized water as different author It was dried, chopped, crushed and powdered with electrical grinder and then the dried powdered samples were stored in polyethylene bottles for further processes.

Phytochemical Determination

Determination of alkaloids

Alkaloids content was measured by following the protocol described by Harborne. A suspension was prepared by dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at 28˚C for 4 hrs which was further filtered through Whatman Number 42.Thereafter alkaloid was precipitated by concentrating the filtrate to one quarter of its original volume and drops of conc. aqueous NH4OH were added. Finally the precipitate was washed with 1% ammonia solution and dried at 80˚C in the oven. The content of alkaloid was calculated and expressed as mg/gm of sample

Determination of riboflavin

To determinine riboflavin, 5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 ml flask, while 10 ml of the extract was put into 50 ml volumetric flask. 10 ml of the 5% potassium permanganate and 10 ml of the 30% H2O2 were added and allowed to stand over a hot water bath for about 30 min. Subsequently, 2 ml of the 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance was measured at 510 nm in a UV/visible spectrophotometer (UV- 1601 SHIMADZU).

Determination of ascorbic acid

Accurately, 1 g of each sample was weighed in a 25 ml conical flask. Then 10 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added and placed in the sample for 24 h, to provide the required reaction time. After 24 h, the samples were filtered through 0.45 µm filter paper. Then 2.5 ml of each sample was transferred to a separate 25 ml volumetric brown flask, after which 2.5 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added. Subsequently, meta phosphoric acid was added separately with acetic acid (0.5 ml), sulphuric acid (5% v/v) solution (1 ml) and ammonium molybdate solution (2 ml) in each volumetric brown flask and the volume was made up to 25 ml with distilled water. The absorbance was measured at 760 nm on a UV/visible spectrophotometer.

Determination of niacin

5 g of the sample were treated with 50 ml of 1 N sulphuric acid and was shaken for 30 min. Three drops of the ammonia solution were added to the sample and was then filtered. Afterwards, 10 ml of the filtrate was added into a 50 ml volumetric flask and 5 ml of 0.02 N H2SO4 absorbance was measured in the spectrophotometer at 470 nm.

Results and Discussion

Medicinal plants are of prime importance to the health of individuals and communities and the medicinal values of these economically important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids and saponin etc. Alkaloids were observed in lower quantity (0.399 mg/100 g) in plant samples in one author, while the other literature found the amount of Alkaloids in medicinal plants by GC-MS method which was 0. 0992 mg/100 g [000]. Other author [500] also detected the high quantity of Alkaloids of 2.667 mg/100 g than the other author. High quantity of flavonoid was detected in, 1.769 mg/100 g. The flavonoids content was quantitatively estimated and was found 0. 373 mg /100 g in other author.

The phenolic content was detected to have high concentration of 1.438 mg/100 g in this author sample analysis, while the other literature found the amount of phenolic content in medicinal plants by GC-MS method was 0. 205 mg/100 g. This variation was may came in case of different methods used and due to the environmental condition in which the plant growth. The lower content of saponins was found in values of 0. 0.09598 mg /100 g in and the amount of saponins 2.516 mg/100 g was recorded to be high in other author (**Table 1**).

Plant name	Alkaloid	Phenol	Flavonoid	Saponin
NB-17	8.62 ± 0.10	22.8 ± 0.004	26.2 ± 0.065	7.65 ± 0.14
Pant Aparna	16.08 ± 0.05	29.4 ± 0.004	63.9 ± 0.061	11.98 ± 0.20
AM-7	4.28 ± 0.30	19.6 ± 0.045	39.1 ± 0.049	13.40 ± 0.30
NB-9	10.7 ± 0.15	10.22 ± 0.032	19.8 ± 0.058	5.36 ± 0.15
Mean	0.0992 ± 0.15	0.020 ± 0.0213	0.373 ± 0.0583	0.096 ± 0.198

Table 1: Quantitative estimation of phytochemicals (mg 100/g).

The biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities. However, alkaloids are mainly observed in large amount in flowering plants and they have an important physiological effect on mankind. Morphine, quinine, ephedrine, nicotine and strychnine are the major types of alkaloids. In these types, morphine and codeine are narcotic analgesics as well as is antitussive agent (**Table 2**)

Plant name	Alkaloid	Phenol	Flavonoid	Saponin
R. arvensis	0.2579 ± 0.007	0.848 ± 0.0072	1.7698 ± 0.022	2.491 ± 0.02
E. ravens	0.3985 ± 0.013	1.068 ± 0.018	1.034 ± 0.008	1.607 ± 0.012
C. lanatus	0.176 ± 0.0013	1.251 ± 0.017	0.5617 ± 0.0013	2.516 ± 0.016
F. critica	0.226 ± 0.0068	1.438 ± 0.013	0.9863 ± 0.0026	0.823 ± 0.004
Mean	0.2646 ± 0.0070	1.151± 0.014	1.0879 ± 0.0085	1.8593 ± 0.013

Table 2: Phytochemicals composition of the four plant samples on dry weight basis expressed as mg/100 g dry weight

Flavonoids are water soluble phytochemical and an important plant phenolic. They show antioxidant activities and they have the property of preventing oxidative cell damage and

carcinogenesis. They have anti-cancer, anti-inflammatory activities and a large effect in lower intestinal tract and heart disease. Flavonoids as antioxidants from R. arvensis, E. ravens, C. lanatus and F. critica provide anti-inflammatory action. These plants show high constitutions of phenols and phenolic compounds which imply that they may be used as anti-microbial agents.

Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides. Thus, due to the hazardous affects as well as antibiotic resistance to the synthetic drugs, researchers are trying to obtain the antimicrobial drugs from medicinal plants due to their non-toxic nature and less side effects. Phenolic compounds have an electron donor capability; moreover, due to this ability, phenolic compounds are readily oxidized to form phenolate ion or quinone, which is an electron acceptor (**Figure 1**).

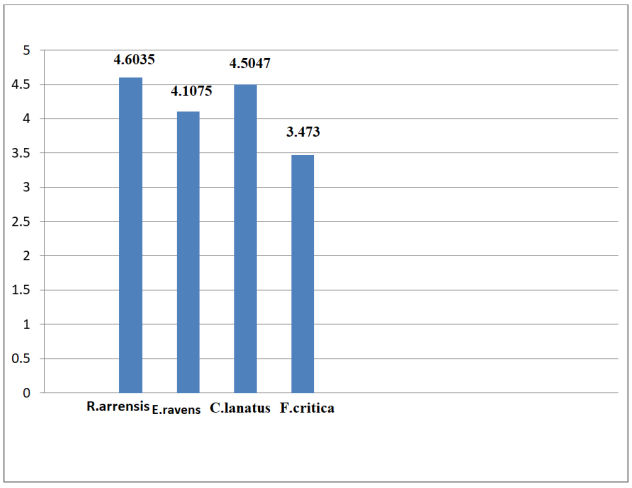

Figure 1: Total amount of phytochemical in the four plant samples.

The comparative values of the total amount of phytochemicals in these plants are shown in **Figure 1**. It is clear from the figure that they are very much rich in phytochemicals such as alkaloids, phenols, flavonoids and saponins

From the over view of the (**Table 1**) and (**Table 2**) there is great difference between these results of the phytochemicals of medicinal plants. So this difference value of results may be due to the environmental factor and condition as well as the methods that researcher used to analysis the phytochemicals of medicinal plants. From the methods, in (**Table 2**), the components of the medicinal plant extracted by methods of methanol extraction, but in ,(**Table 1**) author s tha t usedo analysis the components of the medicinal plant by the methods of GC-MS techniques. When we compared the values, the results are very higher in (**Table 2**) than in the (**Table 1**), this may due to the introduction of the sample to the GC in the volatile gases state which inherent instability of volatile components and losses as well as poor recovery of these substances. This review paper indicates that there are several methods to investigate the components of medicinal plants. As you seen from this review paper, for different methods there is different results, so to compensate this differentiates of the results and to minimized spectral and extraction interference of the result, use of HPTLC-MS method is the best way and more advisable methods of medicinal plant analysis. This review paper gives direction for researchers to analysis the components of the medicinal plants by this methods is better than the other methods.

Conclusion

Medicines derived from plants have made immense contribution towards the betterment of human health and act as a source of inspiration for novel drug compounds. As the review of this paper, there is use of more advanced and sophisticated instrument to overcome several interferences and problems in the analysis of phytochemicals of medicinal plants. Even if there are several methods, HPTLC-MS technique is one of the more advanced and new developed techniques which can be analysis more complex components of the medicinal plants. Another advantage is that the HPTLC system can be easily linked to a scanning densitometer, this not only allows for more precise quantitative work to be carried out but also the data can be exported for multivariate analysis.

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