World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Comparative phytochemical investigations for standardization of some spices available in Pakistan

Najma Shaheen[1], Iqbal Azhar[1], Zeb-un-nisa[2] and Zafar Alam Mahmood[3*]

[1] Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan
[2] Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Pakistan
[3]Colorcon Limited, Flagship House, Victory Way, Crossways, Dartford, Kent, England.

Received: 26-05-2015 / Revised: 21-07-2015 / Accepted: 27-07-2015

ABSTRACT

Spices have been reported to contain a large number of phytochemicals capable of producing color, exceptional taste and aroma, apart from promising biological activity. Being an integral part of culinary, the utilization of standardized spices in various raw and processed food has now been observed to be quite demanding and necessary to obtain desired effects from various spices. Present study deals with the phytochemical investigations of ten different spices commonly available and used in Pakistan with the aim to use the findings as a tool for standardization process. The spices tested include; black pepper, caraway, cardamom large, cardamom small, cinnamon, clove, coriander, cumin seed, red chili and turmeric. The phytochemical screening results of the spices indicated the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, fixed oils, fats, cardiac glycosides, steroids, sterols and terpenoids. The presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, phenols, steroids and sterols, fixed oils and fats were confirmed in all the ten spices used in the study. Saponins and tannins in all spices with the exception of clove and turmeric. Presence of carbohydrates were observed in eight spices except black pepper and cinnamon, while proteins detected in six spices with the exception of cardamom small, cinnamon, clove and coriander. In all spices coumarins were observed except red chili and turmeric. Our analysis confirmed the presence of mucilage in cardamom large, cardamom small, cinnamon, clove and in turmeric only. The phlobatannins results were positive only in black pepper and cardamom large. The presence or absence of various phytochemicals as recoded in the present study were quite comprehensive and trust to provide immense opportunity to draw a guideline to use these findings for the standardization of commercial samples of spices.

Key words: Spices, phytochemicals, phytochemical analysis, standardization

INTRODUCTION

The perception, popularity and general acceptability to use spices in culinary and traditional medical practice has tremendously increased worldwide in recent years. Spices are used alone as well as in combination with certain herbs to achieve desired effect through a balanced formulation, which reflects presence of quite complex chemical composition or entity. Therefore, the process of good quality control / assurance and standardization of spices has now become a current need rather than a desire. A product, thus containing single spice as such or mixture of spices with properly defined and reproducible specifications is now one of the most important prerequisites and primary step for the production of a quality spice. This suggests standardization process to become an integral part to appraise the quality, on the basis of the concentration of active principles present therein. The assurance of the reproducible quality of spices is also helpful in establishing its safety and efficacy. It will not be exaggerated to mention here that, standardization should be treated as an imperative measure to establish high performance, dependable biological activity, a constant chemical profile, or minimally a program relating to quality assurance for production and marketing of spices. Thus, apart from all the qualitative and quantitative methodologies, standardization also requires implementation of GMP at every step. Spices are natural compounds derived from many parts of plants: seed, flowers, fruits, leaves, bark, roots and

***Corresponding Author Address:** Zafar Alam Mahmood, Colorcon Limited, Flagship House, Victory Way, Crossways, Dartford, Kent, England.*

rhizomes etc. Spices not only used for dietary purposes like aroma, color, taste, flavor and preservations of foods but also used as a medicine in traditional system of medicine. Spices have their own unique aroma and flavor which derived from phytochemical compounds [1, 2]. Most phytochemicals are secondary metabolites and have been reported to possess vast biological properties like anticancer, antioxidant, anti-inflammatory, antimicrobial and antidiabetic etc. Further, they may act as detoxifying, neuro-activator and immunity potentiating agents. In addition, some spices may decrease the platelets aggregation and involve in the modulation of hormone. The plant metabolites have a wide range of chemicals with different potency and exhibit more than one function. Around 150 secondary metabolites have been investigated and classified according to their physico-chemical characteristics and protective functions [3]. Therefore, different secondary metabolites reported to be present in spices, such as alkaloids, steroids, tannins, phenolics, flavonoids, sterols, resins and fatty acids can be used as key chemical constituents for the standardization [4].

Based on the objectives of the study, i.e., to standardize the spices through the phytochemical evaluation, present study was taken into consideration and in the initial phase ten different common spices were selected to explore their phytochemical constituents through qualitative reactions or tests. Trust, this will help to exclude adulterations or substitutions and to ascertain the identity and purity of the spices and to establish characteristic specifications and guidelines that can be used to ensure that materials, products, processes and services are all robust for any specific use. The quantitative evaluation of these phytochemicals and identification of some major of key component is under investigations and will be reported in subsequent research publications.

MATERIALS AND METHODS

Selection and identification of plant materials: All the spices named as Black pepper (*Piper nigrum* L.) Family: *Piperaceae*, Caraway (*Carum carvi* L.), Family: *Apiaceae*, Cardamom large (*Amomum subulatum* L.), Family: *Zingiberaceae,* Cardamom small (*Elettaria cardamomum* L.), Family: *Zingiberaceae,* Cinnamon (*Cinnamomum zeylanicum* L.), Family: *Lauraceae,* Clove (*Syzygium aromaticum* L.), Family: *Myrtaceae,* Coriander (*Coriandrum sativum* L.), Family: *Apiaceae*, Cumin seed (*Cuminum cyminum* L.), Family*: Apiaceae*, Red chili (*Capsicum annuum* L.), Family*: Solanaceae* and Turmeric (*Curcuma longa* L.) Family: *Zingiberaceae* were purchased from the local herbal market and identified. Their respective specimen samples are available in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi – Pakistan.

PHYTOCHEMICAL SCREENING

Preparation of samples: The phytochemical screening was performed with the help of powdered material and extract as per recommended method of analysis. Test samples (5.0 g each) were transferred as coarsely powdered to a conical flasks and 50 ml of hexane, ethanol, chloroform, and distilled water were added as single solvent in each flask and stoppered. The mixtures were allowed to stand at room temperature for 24 hours. Finally samples were filtered with the help of Whatman filter paper No 1 and used for various qualitative tests [5].

Detection of Alkaloids: 5ml from each extract was evaporated to dryness and the residue were taken in 5ml of 2% hydrochloric acid (saturated with sodium chloride) in a separate test tube and filtered. The filtrates were used to perform the following test for the detection of alkaloids.

A. Dragendorff's test: Test samples filtrates (2 ml each) were transferred in separate test tubes and 5 drops of Dragendorff's reagent was added into each test tube. The appearance of orange brown precipitates confirmed the presence of alkaloids [6].

B. Wagner's test: Test sample filtrates (2ml each) were transferred in separate test tubes and 5 drops of Wagner's reagent was added into each test tube. The appearance of reddish brown precipitates confirmed the presence of alkaloids [6].

Detection of Carbohydrates

A. Molish's test: Test sample extracts (3 ml each) were transferred in separate test tubes then 10-12 drops of Molish's reagent (α naphthol solution in alcohol) was added into each test tube. The test tubes were shaken well and concentrated sulfuric acid was added along the side of each test tube. Development of violet ring at the junction of two liquids indicated the presence of carbohydrates [7].

B. Fehling's test: 1ml of Fehling's solution A and 1ml of Fehling's solution B were mixed in separate test tubes and boiled for one minute and 2ml of test sample extract was added. The content was heated in boiling water bath for 5-10 minutes. (The same test was repeated for each sample extracts). Development of yellow color (initially) followed by a brick red precipitate indicated the presence of carbohydrates [7].

Detection of Flavonoids

A. Color test: 0.5g powdered material from each test sample was taken in separate test tubes and 10ml of hexane, ethanol, chloroform and distill water were added as a single solvent in each test tube and were heated on a water bath for 3 min. The mixture was filtered in another test tube and from this 4 ml of each filtrate was transferred in separate test tube and 1 ml of dilute ammonia solution was added and shaken. Appearance of yellow color was noted as presence of flavonoids [8].

B. Lead acetate test: Test sample extracts (1ml each) were transferred in separate test tubes and 12-15 drops of the lead acetate solution was added in each test sample. Formation of yellow color precipitate was recorded as presence of flavonoids [9].

Detection of Fixed oil and Fats

A. Spot test: 0.1g of each test sample extract was pressed separately between filter paper and the paper was observed and matched with the control, which was performed by placing 2 drops of olive oil on filter paper in a similar way. Appearance of translucency of the filter paper confirmed the presence of fixed oil and fats [10].

B. Saponification test: 5ml of the each test sample extract was transferred in separate test tubes. 20 drops of 40% NaOH and 2 ml of glycerol were added in each test tube and gently boiled for 3 minutes until complete saponification occurred. Oil globules were observed upon continuous boiling and at this stage the solution was divided in to 3 parts to perform following experiments in test tubes 1, 2, 3 of each test sample extracts. In test tube no.1, 5 to 6 drops of saturated NaCl was added to separate the soap which will then float on the surface. In test tube no.2, 5 to 6 drops of concentrated HCl was added to raise the oily layer of the fatty acids. In test tube no.3, 5 to 6 drops of $CaCl_2$ solution was added to precipitate the calcium soap. Results were observed carefully and recorded [11].

Detection of Cardiac glycosides

A. Keller-Killiani test: Test sample extracts (2ml each) were transferred in separate test tubes and 1 ml glacial acetic acid, one drop 5% $FeCl_3$ and 1ml concentrated H_2SO_4 were added in each test tube. Appearance of reddish brown color at the junction of two liquid layers, while appearance of bluish green color at the upper layer indicated the presence of cardiac glycosides. [7].

B. Legal's test: Test sample extracts (1ml each) were taken in separate test tubes and 1ml pyridine and 1ml sodium nitroprusside were added in each test tube. Development of pink to red color confirmed the presence of cardiac glycosides [7].

Detection of Resins

A. Precipitate test: The test sample extracts (0.2g of each) were transferred in separate beakers and each test sample extract was treated with 15ml of 96% ethanol. The content was poured into 20 ml of distilled water in separate beaker and repeated for each test sample. Formations of precipitates indicated the presence of resins [10].

B. Color test: Test sample extracts (0.12g of each) were transferred in separate test tubes and 1ml of chloroform was added in each test tube and the extracts were concentrated to dryness. The residues were re-dissolved in 3ml of acetone and 3 ml of concentrated hydrochloric acid was added into each tube. The tubes were then heated in a water bath for 30 minutes. Appearance of pink color initially, followed by development of magenta-red color confirmed the presence of resins [10].

Detection of Phenols

A. Ferric Chloride test: Test sample extracts (1 ml each) were transferred in separate test tubes and 3-4 drops of ferric chloride solution was added in each test tube. Appearance of bluish black color indicated the presence of phenols [9].

B. Lead acetate test: Test sample extracts (1ml each) were transferred in separate test tubes and 5-6 drops of lead acetate solution was added in each test tube. Formations of white precipitates revealed the presence of phenols [7].

Detection of Saponins

A. Froth test: 1g of the powdered each test sample was transferred in separate test tubes and 10 ml of hexane, ethanol, chloroform and distilled water were added as a single solvent in each test tube. The contents of each test tubes were boiled in a water bath and then filtered. After filtration 5 ml each filtrate was transferred in a separate test tube and 2.5ml of distilled water was added, shaken vigorously and observed for a stable persistent forth. The frothing was mixed with 2 drops of olive oil and shaken vigorously. The formation of emulsion was recorded as positive saponins test. [8].

B. Foam test: 0.5 gm of test sample extracts were transferred in separate test tubes and 2ml of distilled water was added in each test tube and then shaken. Formation of foam which persists for at least 10 minutes, indicated the presence of saponins [9].

Detection of Steroids and Sterols

A. Salkowski reaction: Test sample extracts (2ml each) were transferred in separate test tubes and

2ml chloroform and 2ml of concentrated sulfuric acid were added in each test tube. The test tubes were then shaken well. Appearance of chloroform layer as red and acid layer as greenish yellow fluorescence confirmed the presence of steroids and sterols [7].

B. Liebermann-Burchard reaction: Test sample of extracts (2 ml each) were transferred in separate test tubes. 2ml chloroform, 2ml of acetic anhydride and 2 drops of concentrated sulfuric acid were added from the wall of each test tube. Appearance of first red, followed by blue and finally green color confirmed the presence of steroids and sterols [7].

Detection of Tannins

A. Ferric chloride test: 0.5g of the dried powered of each sample was taken in separate test tubes and 20 ml of hexane, ethanol, chloroform and distilled water were added as single solvent in each test tube and boiled on a water bath and then filtered. After filtration 6 drops of 0.1% ferric chloride was added in each test tube. Appearance of blue-black coloration revealed the presence of tannins [8].

B. Lead acetate test: Test sample extracts (1ml each) were transferred in separate test tubes and 3 drops of the lead acetate solution was added in each test tube. Formation of cream gelatinous precipitates confirmed the presence of tannins [10].

Detection of Terpenoids

A. Salkowski test: Test sample extracts (5ml each) were transferred in separate test tubes and 2 ml of chloroform, and 3ml of concentrated sulfuric acid were added carefully to form a layer. Formation of a reddish brown coloration at the interface showed positive results for the presence of terpenoids [8].

B. Color test: Test sample extracts (0.4g each) were transferred in separate test tube and 0.5ml of acetic anhydride and 0.5 ml of chloroform were added in each test tube. It was followed by the addition of concentrated solution of sulfuric acid. Appearance of red violet color indicated the presence of terpenoids [12].

Detection of Mucilage

A. Color test: 0.2g of each test sample extracts were transferred in separate test tubes and 1 drop of iodine solution with 2 drops of H_2SO_4 were added in each test tube. Development of violet color showed the presence of mucilage [7].

B. Swell test: 0.5g of each powdered test sample was taken in separate petri dish and mixed with distilled water. Swelling of powder after few minutes recorded as positive test for the presence of mucilage [7].

Detection of Phlobatannins: Test sample extracts (5 ml each) were transferred in separate test tubes and 4 ml of 1% aqueous hydrochloric acid was added in each test tube and boiled on a water bath. Formation of red precipitates in samples which revealed the presence of phlobatannins [8].

Detection of Proteins

Xanthoprotein test: Test sample extracts (3ml each) were taken in separate test tubes. 1 ml of concentrated sulfuric acid was added along the sides of each test tube. Formation of yellow precipitate as positive test for the presence of proteins [13].

Detection of Coumarins: 0.5g moistened dry powder of each test sample were taken in separate test tubes. The test tubes were covered with filter paper, already soaked in dilute NaOH. The tubes were kept in water bath. The filter papers were then exposed to UV light and observed for color development. Appearance of yellowish green fluorescence indicated the presence of coumarins [7].

RESULTS AND DISCUSSION

The phytochemical screening of all these spices was qualitatively analyzed and their results are shown in table-1. In the present study alkaloids, flavonoids, fixed oils, fats, glycosides, resins, phenols, terpenoids, steroids and sterols were noted to present in all four (hexane, chloroform , ethanol and distill water) extracts of tested spices, while in black pepper extracts, carbohydrate and mucilage were absent. Cinnamon extracts also showed the absence of carbohydrates, phlobatannins and proteins, whereas clove extracts showed absence of saponins, phlobatannins and proteins. In turmeric tannins, phlobatannins, and coumarins were absent. Caraway showed negative result for mucilage and phlobatannins, while cardamom small indicated the absence of phlobatannins and proteins, coriander showed absence of mucilage, phlobatannins and proteins. Cumin seed indicated absence of mucilage and red chili showed negative result for mucilage, phlobatannins and coumarins. It is expected that the identification of these phytochemicals will help to draw a guidelines to ascertain the quality of these tested spices.

Phytochemicals detected in these spices, possess various biological activities which may help in fighting against chronic diseases for example alkaloids have anti-microbial, anti-hypertensive, anti-arrhythmic, anti-malarial, and anti-cancer effects. Tannins in traditional medicine used against diarrhea, duodenal ulcer, anti-

inflammatory, anti-septic, hemostatic, astringent and as diuretic. Spices are also enriched in flavonoids (hydroxyl phenolic compounds) showing the activities, such as anti-microbial, anti-oxidant, anti-tumor, anti-inflammatory, estrogenic, anti-allergic, enzyme inhibition and vascular activities. Terpenoids have been reported to possess anti-carcinogenic, anti-ulcer, anti-malarial, and anti-microbial activities. Saponins have been noted as anti-oxidant, anti-microbial, and hypoglycemic in activities. Cardiac glycosides used as anti-arrhythmic agents and the treatment of proliferative diseases as well as for the inhibition of several cancers, such as lung, prostate, breast and leukemia. Steroids have anti-bacterial activity; regulate immune response and cholesterol-reducing property. Carbohydrates or reducing sugar known as main fuel in biological system and provide with useable energy to living cells. Coumarins are naturally substance and show anti-oxidant, anti-coagulant, anti-inflammatory and analgesic, anti-viral, anti-cancer, anti-malarial and anti-microbial activities [3, 14, 15, 16, and 17]. Phenolic compounds exhibit anti-oxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory activities [18]. In our study, the results showed that the identified phytochemical compounds in the tested spices may play an important role in the management and treatment of some diseases as well along with their culinary applications. Therefore, use of standardized spices alone or in combination is well justified to achieve the desired effect in foods as well as therapeutic effect if used for curative purpose. The process of standardization is necessary to achieve the authenticity of spices. The process of standardization helps in correct identification and authentication of spices, minimizing the contamination that may occur due to lack of experience and information relating to difference in geographical states and local names. Only a small percentage of spices have reported to be investigated phytochemically, therefore trust the results of the present study will be helpful assessing and stablishing the quality of spices available in local market. In addition, the ongoing quantitative analysis, once available will further support and authenticate our quality control proposals based on phytochemical analysis.

CONCLUSION

Phytochemical investigation is now been considered as one of the leading tool in the standardization process of plants and plant based products. As their presence or absence has significant impact on the quality and purpose of use, therefore, it is believed that their studies will be helpful in the standardization of spices as well. In the present study, the phytochemical screening, based on the detection of terpenoids, flavonoids, steroids, cardiac glycosides, proteins, saponins, tannins, mucilage, resins, alkaloids, phenols, fixed oil, fats, phlobatannins and reducing sugars provided reasonable data to establish the quality of spices available in Pakistan market. Since, most of these phytochemicals are classified as secondary metabolites, therefore, their studies will also be helpful in understanding the additional properties of these selected spices which are used for their medicinal purpose as well. The presence of these secondary metabolites have linked to various pharmacological properties of spices, such as anti-microbial, analgesic, anti-malarial, anti-inflammatory, anticancer, and diuretic activities etc. The quantitative estimation of these phytochemicals are under evaluation and once completed will be published in due course to further support the standardization process of spices with reasonable justification to establish upper and lower limits of acceptance. Hope, the criteria established for the standardization of spices using these phytochemicals will also provide guidelines for the manufacturers as well to adopt in their standard operating procedures while handling issues of quality of common spices.

Table: 1 Results of preliminary screening of primary and secondary metabolites in ten spices

S. no	Secondary metaboliles	Name of test	Solvent used	1	2	3	4	5	6	7	8	9	10
1	Alkaloids	Dragendorff's test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+++	+	+	+	+	+	+
			IV	+	+	+	++	+	+	+	+	+	+
		Wagner's test	I	+	+	+	+	+	+	+	+	++	+
			II	+	+	+	+	+	+	++	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	++	+	+	+
2	Carbohydrates	Molish test	I	-	+	+	+	-	+	+	+	+	+
			II	-	+	+	+	-	+	+	+	+	+
			III	-	+	+	+	-	+	+	+	+	+
			IV	-	+	+	+	-	+	+	+	+	+
		Fehling's test	I	-	+	+	+	-	+	+	+	+	+
			II	-	+	+	+	-	+	+	+	+	+
			III	-	+	+	+	-	+	+	+	+	+
			IV	-	+	+	+	-	+	+	+	+	+
3	Flavonoids	Color test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
		Lead acetate test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	++	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+++	++	+++	+
4	Fixed Oil and Fats	Spot test	I	+	++	+	+	+	+++	+	+++	+	+
			II	+	+	+	+	+	+	+	+	++	+
			III	+	+++	+	+	+	++	+	++	+	+
			IV	+	+	+	+	+	+	+	+	+	+
		Saponification test	I	+	+	+	+	+	+	+	++	++	+
			II	+	+	+	+	+	+	+	++	++	+
			III	+	+	+	+	+	+	+	++	++	+
			IV	++	+	+	+	+	+	+	++	++	+
5	Cardiac glycosides	Keller killiani test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
		Legal test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
6	Resins	Precipitate test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
		Color test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	x	x	x	x	x	x	x	x	x	x
7	Phenols	Ferric chloride test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
		Lead acetate test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+

Table: 1 (Continued)

8	Saponins	Froth test	I	+	+	+	+	+	-	+	+	+	+
			II	+	+	+	+	+	-	+	+	+	+
			III	+	+	+	+	+	-	+	+	+	+
			IV	+	+	+	+	+	-	+	++	++	+
		Foam test	I	+	+	+	+	+	-	+	+	+	+
			II	+	+	++	+	+	-	+	+	++	+
			III	+	+	+	+	+	-	+	+	+++	+
			IV	+	++	++	+	+	-	+	+	++	+
9	Steroid and Sterol	Salkowski test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
		LIberman-buchard test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	+	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	+	+	+	+	+	+
10	Tannins	Ferric chloride test	I	+	+	+	+	+	+	+	+	+	-
			II	+	+	+	+	+	+	+	+	+	-
			III	+	+	+	+	+	+	+	+	+	-
			IV	+	+	+	+	+	+	+	+	+	-
		Lead acetate test	I	+	+	+	+	+	+	+	+	++	-
			II	+	+	++	++	+	++	++	++	++	-
			III	+	+	+	+	+	+	+	+	+	-
			IV	+	+	++	++	+	++	++	++	++	-
11	Terpenoids	Salkowski test	I	+	+	+	+	+	+	+	+	+	+
			II	+	+	+	+	++	+	+	+	+	+
			III	+	+	+	+	+	+	+	+	+	+
			IV	+	+	+	+	++	+	+	+	+	+
		Color test	I	+	++	++	++	++	++	++	++	++	++
			II	+	++	++	++	++	++	++	++	++	++
			III	+	++	++	++	++	++	++	++	++	++
			IV	+	+	+	+	+	+	+	+	++	+
12	Mucilage	Swelling test		-	-	+	+	+	+	-	-	-	+
		Color test	I	-	-	++	++	++	+	-	-	-	++
			II	-	-	+	+	+	+	-	-	-	+
			III	-	-	+	+	++	++	-	-	-	+
			IV	-	-	+	+	+	+	-	-	-	+
13	Pholbatanins	Precipitate test	I	+	-	+	-	-	-	-	-	-	-
			II	+	-	+	-	-	-	-	-	-	-
			III	+	-	+	-	-	-	-	-	-	-
			IV	+	-	+	-	-	-	-	-	-	-
14	Protein	Xanthoprotein test	I	+	+	+	-	-	-	-	+	+	+
			II	++	++	+	-	-	-	-	+	+	+
			III	+	+	+	-	-	-	-	+	+	+
			IV	+	+	+	-	-	-	-	+	+	+
15	Coumarin	Fluorescence test		+	++	+	+	++	++	++	+	-	-

I = Hexane extract, II = Ethanol extract, III = Chloroform extract, IV = Distill water extract, 1 = Black pepper, 2 = Caraway, 3 = Cardamom large, 4 = Cardamom small, 5 = Cinnamon, 6 = Clove, 7 = Coriander, 8 = Cumin seed, 9 = Red chili, 10 = Turmeric + = Low concentration, ++ = Moderate concentration, +++ = High concentration, - = Absent, x = Not used

Figure: 1 Selected spices for the phytochemical investigations

Black Pepper

Coriander

Cardamom large

Cumin seed

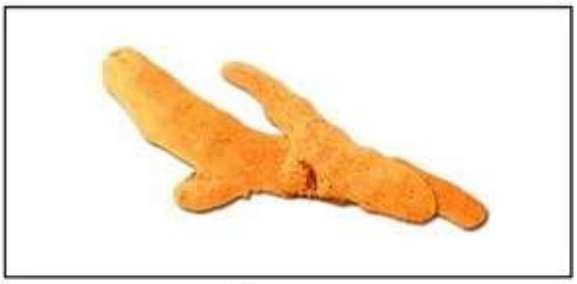
Cardamom small

Clove

Red chili

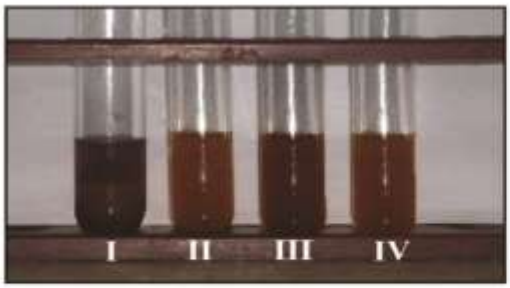
Cinnamon bark

Caraway

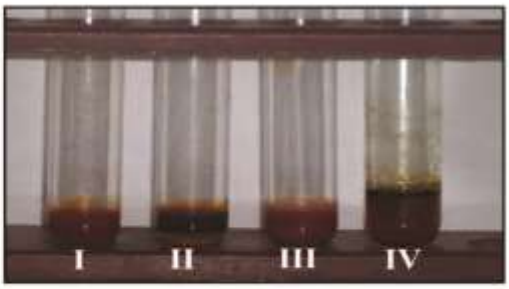


Turmeric

Figure: 2 Results of the phytochemical screening test



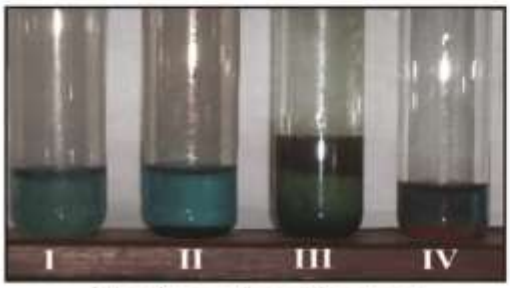
Alkaloids test



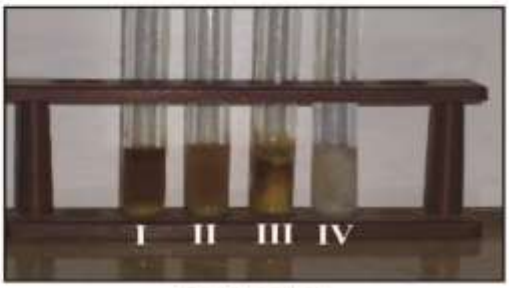
Carbohydrates test

Flavonoids test

Fixed oil and fats test



Cardiac glycosides test

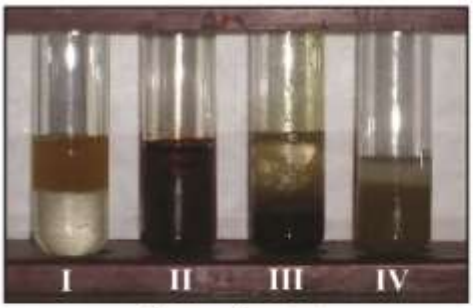


Resins test

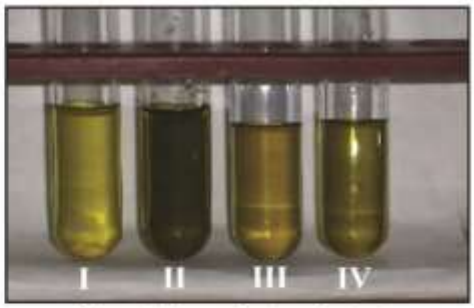
Phenols test

I. Hexane extract, II. Ethanol extract, III. Chloroform extract, IV. Distill water extract, Alkaloids test: Reddish brown precipitate, Carbohydrates test: Brick red precipitate, Flavonoids test: Yellow color, Fixed oil and fats Test: soap layer, oily layer and precipitate, Cardiac glycosides test: Reddish brown and bluish green color, Resins test: Appearance of precipitate, Phenols test: Bluish black color

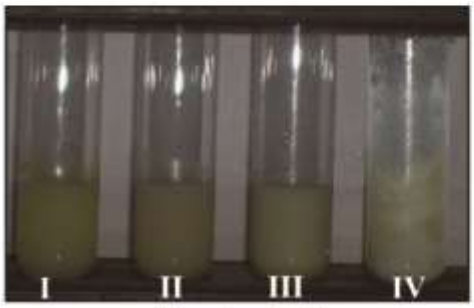
Figure: 2 (Continued)



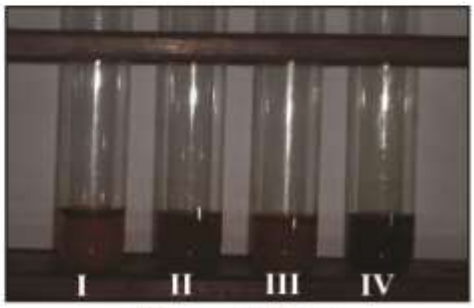
Saponins test



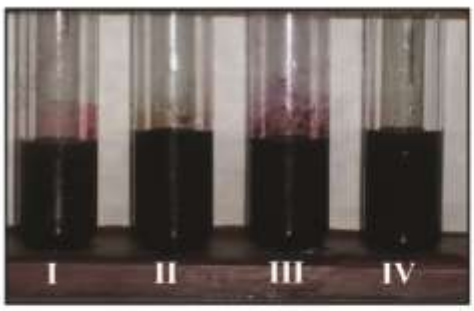
Steroids and sterols test



Tannins test

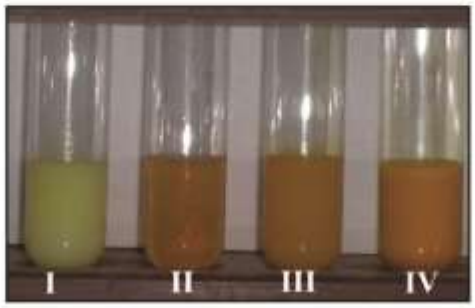


Terpenoids test



Mucilages test

Phlobatanins test



Proteins test

Saponins test: Appearance of foam, Steroids and sterols test: Green color, Tannins test: Cream gelatinous precipitate, Terpenoids test: Reddish brown color, Mucilage test: Violet color, Phlobatannins test: Red precipitate, Proteins test: Yellow precipitate.

REFERENCE

1. Ramaya BS, Ganesh P. Phytochemical analysis and comparative effect of *Cinnamomum zeylanicum, Piper nigrum* and *Pimpinella anisum* with selected antibiotics and its antibacterial activity against enterobacteriaceae family. International Journal of Pharmaceutical & Biological Archives, 2012; 3(4): 914-917.
2. Prasad MP et al. Phytochemical analysis and antioxidant potential of *Piper* species and its Molecular Characterization by RAPD Markers. International Journal of Fundamental &Applied Sciences. 2012; 1(4): 71-73.
3. Saxena M et al. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013; 1(6): 168-182.
4. Swadhini SP et al. Phytochemical screening and antimicrobial activity of five medicinal plants against Myrothecium SP. International Journal of Pharma and Bio Sciences. 2011; 2(1): 272-279.
5. Ugochukwu SC et al. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian Journal of Plant Science and Research. 2013; 3(3):10-13.
6. Kumar U et al. Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom. International Journal of pharmaceutical science and research, 2010; 1(12): 138-147.
7. Khandelwal KR. *Practical pharmacognosy techniques and experiments*. 18th ed; Nirali Prakashan. Pune, 2007.
8. Edeoga HO et al. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4(7): 685-688.
9. Tiwari P et al. Phytochemical screening and extraction: A Review. Inernational Pharmaceutica Sciencia. 2011;1(1):98-106.
10. Ukoha PO et al. Tannins and other phytochemical of the *Samanaeasaman* pods and their antimicrobial activities. African Journal of Pure Applied Chemistry. 2011; 5(8): 237-244.
11. Kalaiarasan A, John SA. Phytochemical screening and antibacterial activity of *Sidacordifolia* L. (Malvaceae) leaf extract. International Journal of Medicibiological Research. 2010;1(2):94-98.
12. Chhetri HP et al. Phytochemical and antmicrobial evaluations of some medicinal plants of Nepal. Khatmandu University Journal of Science, Engineering and Technology. 2008;1(v):49-54.
13. Raju B et al. Evaluation of cardiotonic activity of *peltophorumpterocarpum*. International Journal of Phytopharmacology.2011;2(1): 1-6.
14. Madhukar C. Phytochemical screening of cumin seed extract. Report and Opinion. 2013; 5(1):57-58
15. Yadav M et al. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. International journal of Pharmaceutical Sciences. 2014;6(5):539-542.
16. Joy A, Alam Md A. Quantitative structure activity relationship (QSAR) of cardiac glycosides: the development of predictive *in vitro* cytotoxic activity model. Der Pharmacia Lettre. 2012;4(4):1246-1269.
17. Sahoo SS et al. Synthesis of novel coumarin derivatives and its biological evaluations. European Journal of Experimental Biology. 2012; 2(4):899-908.
18. Latha R et al. Physicochemical and phytochemical investigation on medicinal plants used by ethnic tribes of Nilgiri Mountains, South India. World Journal of Pharmaceutical Sciences. 2015; 3(5):632-644.