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Extraction of *Momordica charantia, Pongamia glabra* and *Piper nigrum*: Qualitative and Quantitative assessment

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ABSTRACT

Objective: The objective of present work was to evaluate the qualitative and quantitative assessment of *momordica charantia, pongamia glabra and piper nigrum extract for the natural product present in them.* These plants were having the rich source of alkaloid, glycoside, tannins, carbohydrates, saponins, flavonoids, proteins and amino acids and were used as anti-diabetic, anti-inflammatory, antitumor, anti-malarial and having wound healing potential. **Materials and Methods:** Extract of all three plants has been separated by the process of Soxhlet extraction. The extract of *momordica charantia, pongamia glabra and piper nigrum* evaluated for qualitative and quantitative measurement of alkaloid, glycoside, tannins, carbohydrates, saponins, flavonoids, proteins and amino acids content. Different solvent is used for the extraction of content includes petroleum ether, ethyl acetate, alcoholic and distilled water. **Results and Conclusion:** Preliminary Phytochemical screening was performed for extracts of *Momordica charantia* fruits (FMC), *Pongamia glabra* (LPG) and *Piper nigrum* fruits (FPN). Identification test on extracts was shown the sign of alkaloid, glycoside, tannins, carbohydrates, saponins, flavonoids, proteins and amino acids content. **Discussion:** All results indicates that extracts of *Momordica charantia* fruits (FMC), *Pongamia glabra* (LPG) and *Piper nigrum* fruits (FPN) having a rich source of Glycosides, alkaloid and flavonoid content.

Keywords: *momordica charantia, pongamia glabra, piper nigrum,* glycosides, flavonoid, extraction.

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INTRODUCTION

*Pongamia glabra (*Figure 1*)* belong to *Fabaceae* family[1]. Fruit contains furano-flavonoids, coumestan and pongacoumestan. *P. glabra* has been reported to contain a large number of furano flavonoids e.g. karanjin, pongapin, kanjone, pongamol. It used as bacteriocidal activity against V. cholerae and E. coli, and also used as anti-inflammatory and antipyretic properties[2]

Piper nigrum (Figure 2) belonging to family *piperaceae*. The fruits have small globose drupe and was known as a peppercorn when dried. Pepper plants grow easily in the shade and require little maintenance until harvest, so they are frequently cultivated for supplemental income on even small farms. Black pepper is used to improve digestion, stimulate appetite and treat gastrointestinal problems. It is also used to treat cold, cough and sore throats. Antioxidant active chemicals isolated from black pepper includes camphene, carvacrol, eugenol, myrcene myristic-acid, myristicin, palmitic-acid and ubiquinone and were responsible for aroma, pungency and medicinal property of the black pepper[3].

Figure 1: Leaves, flowers and seeds of *Pongamia glabra*

Figure 2: Unripe and ripe seeds of *Piper nigrum*

Momordica charantia belong to Cucurbitaceae family[4]. The fruit has a distinct warty looking exterior and an oblong shape. It is hollow in cross section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds and pith. (Figure 3). *M. Charantia* consists the following chemical constituent charantin, diosgenin, gentisic acid, myristic acid and nerolidol. *Momordica charantia* is used as anthelmentic, anti-mycobacterial, antioxidant, antitumor, wound healing prpperties, antiulcer, antiviral, hypoglycemic and immune-stimulant[5].

Figure 3: Fruit of *M. Charantia*

The objective of the present paper was to evaluate the constituent present in *momordica charantia, pongamia glabra and piper nigrum* extract. Preliminary test has been performed for the measurement of the constituents present in all three plants. Test for alkaloids, alkaloid, glycoside, tannins, carbohydrates, saponins, flavonoids, proteins and amino acids were performed.

MATERIAL AND METHOD

Drugs are collected from wild plants or cultivated plants. The season in which the drug is collected plays an important role in determining the quality of drug. Organoleptic characters, morphological characters and microscopically examination would help in identifying crude drug. Generally, three methods are employed in the extraction of plant materials as (1) Maceration (2) Percolation (3) Soxhlet extraction. Maceration and percolation may be employed in extraction of thermo labile constituents. Soxhlet extraction is rapid and continuous and may be employed in extraction of sparingly soluble constituents due to repeated extraction, which cannot be done by either maceration or percolation methods. Soxhlet extraction process was use for present study.

Plant material collection and authentication

The fruits of *Momordica charantia* were collected at in the month of July from local field areas of Bhopal region, M.P., Leaves of *Pongamia glabra* from Garden and fruits of *Piper nigrum* from local market of Bhopal, Madhya Pradesh. The specimens were submitted and identified as fruits of *Momordica charantia* (MC) family of Cucurbitaceae, Leaves of *Pongamia glabra* (PG) family of Fabaceae and fruits of *Piper nigrum* (PN) family of Pipereacea and authenticated by Dr. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal. The appession no. for the specimen is 490/BS/saifia/16 has been preserved for future identification. These samples were shade dried so as to protect its chemical constituents not to get degrade at high temperature.

Morphological and physicochemical properties

These parameters involve the determination of ash values, foreign matter, extractable matter, volatile oil content of the preparations or individual drugs[6]. These parameters give the idea of the physical characteristics and the chemical constituents present in extract of all three plants.

Physical Evaluation

Determination of Foreign Matter

100 gm of the sample was weighed and spread on a white tile uniformly without overlapping. Then the sample was inspected by means of 5x lens and the foreign organic matter was separated. Then after complete separation, matter was weighed and percentage w/w was determined.

Solvent Extractive Values:

In this water soluble and alcohol soluble extractive value has been identified by followed procedure.

Determination of Water-soluble extractive value

5 gm of powdered drug was macerated with 100 ml of distilled water in round bottom flask for 24 h and was occasionally shaking for 6 h. Then after allowed to stand for 18 h. Filter out the solution and evaporated to dryness in a tarred flat bottom shallow dish. Dry at 105^{o}C and weighed. Percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Determination of Alcohol soluble extractive value

5 gm of powdered drug was macerated with 100 ml of ethanol in round bottom flask for 24 h and was occasionally shaking for 6 h and was allowed to stand for 18 h. After filtration, the filtrate was evaporated to dryness in a tarred flat bottom shallow dish. Dry at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

Determination of total ash

Total ash was determined by weighing 2-3gm of the air-dried crude drug in the tared platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then was cooled and weighed.

Determination of acid insoluble ash

Ash insoluble in HCI is the residue obtained after extracting the sulfated or total ash with HCI and calculated with reference to 100gm of drug. The ash obtained from the previous process was boiled with 25ml of 2M HCI for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a desiccator and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of water-soluble ash

The ash was boiled with 25ml of water for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 15min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug.

Successive solvent extraction

Successive Soxhlet extraction by using solvents, selected on the basis of polarity after defatting of the crude drug by petroleum ether. Solvents used were ethyl acetate, ethanol, and water.

Soxhlet Extraction

The crude drugs were dried in shade. Then moderately coarse powder of the drugs e.g. *Momordica charantia* (MC), *Pongamia glabra* (PG) and *Piper nigrum* (PN) were subjected to successive Soxhlet extraction with different solvents in increasing order of polarity from non-polar to polar. Successive Soxhlet extraction is rapid, continuous and may be employed in sparingly soluble constituent due to repeated extraction, which cannot be done by either percolation or maceration methods.

Extraction was performed by following procedure includes by taken 80 gm of dried coarsely powdered drug and packed in Soxhlet apparatus and defatted with 1000 ml of petroleum ether at 40-60°C until the complete defatted. Complete defatting ensured by placing a drop by thimble on the filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove the last traces of petroleum ether. The defatted material was subjected to extraction by Ethyl acetate then with Ethanol as solvent by Soxhlet apparatus and finally with water by maceration process. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent.

The marc was air dried before extracted with the next solvent. Dried marc was macerated with water for 24 h. The extracts were evaporated under reduced pressure at low temperature (30°C) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies. They were weighed to a constant weight and percentage w/w basis was calculated[7].

Preliminary phytochemical screening

Qualitative Test Analysis

Dried extracts were taken for the chemical test for detection of the phytoconstituents like alkaloids, flavonoid, tannins, sterols, phenolic compounds, terpenoids, carbohydrates etc. In order to detect the various constituents, present in the different extracts of MC, PG and PN. Those were subjected to the tests as per methods[8.9.10].

Quantitative Analysis

Determination of Total Phenolic Content

The total phenolic content of all the extracts was determined by using Folin-Ciocalteu method. A standard gallic acid curve was constructed by preparing the dilutions of (0.8, 0.6, 3.12, 6.25, 12.5 and 25 µg/ml) in methanol from standard solution of gallic acid. 100µl of each of these dilutions were mixed with 500µl of water and then with 100µl of Folin-Ciocalteu reagent and allowed to stand for 6 minutes. Then 1ml of 7% sodium carbonate and 500µl of distilled water was added to the reaction mixture. The absorbance was recorded after 90 minutes at 760 nm, through UV-spectrometer. The same procedure was repeated with extracts. The total phenolic content of the extracts was calculated as gallic acid equivalents (mg GAE/g)[11]. All the experiments were performed in triplicate. All experimental measurements were carried out in triplicate and are expressed as average of three analyses. The magnitude of correlation between variables was done using a Microsoft excel.

Determination of total flavonoid content

Quercetin was used as standard and flavonoid content was determined as quercetin equivalent. A calibration curve for quercetin was drawn for this purpose. From the standard quercetin solution, and make dilutions of (10, 20, 30, 40 and 50 µg/ml) concentrations were prepared in methanol. 100µl of each of the quercetin dilution was mixed with 500µl of distilled water and then with 100µl of 5% Sodium nitrate and allowed to stand for 6 minutes. Then 150µl of 10% aluminum chloride solution was added and allowed to stand for 5 minutes after which 200µl solution of 1M sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510nm on UV spectrophotometer[11]. The same procedure was repeated with the extracts and total flavonoid content was calculated as quercetin equivalents (mgQE/g). All the procedures were performed in triplicate. All experimental measurements were carried out in triplicate and are expressed as average of three analyses. The magnitude of correlation between variables was done using a Microsoft excel.

RESULTS AND DISCUSSION

Morphological and physicochemical properties

The immature fruit of *Momordica charantia* are green, pulp pithy, whitish yellow at maturity exposing numerous seeds, enclosed in whitish aril which becomes bright red on maturity. The seeds are bitter, pale brown up to 1.5 cm long, flattened, elliptic with scalloped markings on the flat

side and on the edge of seed. The microscopy of the *Momordica charantia* fruits shows the presence of epicarp, hypodermis, middle mesocarp, vascular bundles, prismatic calcium oxalate crystals, seeds, aril & inner mesocarp and the seed shows tesla, endosperm, cotyledon, perisperm.

The *Piper nigrum* fruits are globular in shape, 3-6mm in diameter. The external surface is dark brown or grayish black and strongly reticulated wrinkled with remains of stigma at apex. The organoleptic evaluation of the fruit and fruit powder revealed that both were greyish black or dark brown in color, with aromatic odor and pungent taste. Transverse section of *Piper nigrum* fruit shows a well-differentiated thick pericarp, testa and inner mass of perisperm and enclosing a small embryo. Pericarp consists of an external epicarp, a large parenchymatous meso carp and a single layer of endocarp containing stone cells. They are varying in shape and size, usually poly gonal to rectangular.

The *Pongamia glabra* leaves are glossy dark green upper surface, dull green lower surface, with characteristic odor. They are ovate or elliptical in shape with 6.2 to 11.5 cm long and 8.3cm wide. They have smooth texture with alternate arrangement.

Physicochemical properties of drugs

Temperature and solvent variation can give impact on the quantity of extractable matter of a plant. The extractive capacity or extractive value increases with the amount of extractive matter produced under a particular condition. The herbal monograph specified that the limits for water soluble extractive values for black pepper are not less than 3% and 2%, respectively for water and alcohol soluble extractive values. All specimens were found superior than the standard specifications. From this study, higher temperature and using water as solvent exhibited a better extractive capacity than in room temperature and alcohol used as solvent. The water-soluble extractive value indicated the presence of sugar, acids and inorganic compounds; the water-soluble extractive value found to be 30.90±0.53, 13.9±0.24 and 10.14±0.06 %w/w for MC, PG and PN respectively. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The alcohol soluble extractive value was found to be 13.86±0.12, 4.31±0.25 and 12.2. ±0.08 %w/w for MC, PG and PN which signify the nature of the phytoconstituents present in plant.

Table 1: Physical characteristics

S. No	Name of the drug	Values	Foreign organic matter	Total ash value	Acid insoluble ash value	Water soluble ash value
1.	*Momordica charantia (mc)*	Theoretical	Nil	<6%	<4%	-
		Observed	Nil	6.82±0.015%	1.04±0.042%	3.49±0.101%
2.	*Pongamia glabra (pg)*	Theoretical	<2%	<5%	<1%	-
		Observed	0.58±0.072%	7.87±0.084%	0.71±0.037%	3.04±0.071%
3.	*Piper Nigrum (PN)*	Theoretical	<2%	<3%	<2%	-
		Observed	1.30±0.024%	2.13±0.044%	1.02±0.084%	0.87±0.077%

Evaluation of crude drug ensures the identity of drug and determines the quality and purity of drugs. The main reason behind the need for the evaluation of crude drug is biochemical variation in the drug, effect of treatment, storage of drug, adulteration and substitutions. The results of the physicochemical parameters of fruit powder depicted within the limit which is mentioned in **Table 1**. The results of foreign organic matter denote presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned Indian Pharmacopeia 1996; which was found to be 0, 0.58±0.072 and 1.30±0.024% w/w for MC, PG and PN respectively. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. All the individual drugs were found to have total ash values in the range from 2.13 to 7.87% w/w **[Table 1]**.

The MC, PG and PN have 6.82±0.015, 7.87±0.084 and 2.13±0.044% w/w total ash values. These values were found to be reasonably low indicating low contamination. Total ash values of the of the individual drugs match up the standard values. Water-soluble ash is the part of the total ash content, which is soluble in water. It is a good indicator of either previous extraction of water-soluble salts in the drug or incorrect preparation. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water. The water-soluble ash values of the individual drugs were in the range of 0.87 to 3.49% w/w. This shows a normal quality of the drugs. Water-soluble ash values of the MC, PG and PN were found to be in the range of 0.87 to 3.49% w/w **[Table 1]**. These values match with the standard average water-soluble extractive values of individual drugs also.

Extraction

The extraction was done by successive solvent extraction, to increase the extraction, to achieve separation of compounds in different extracts and decrease the time taken by extraction process the flask and Soxhlet apparatus was covered by cotton to increase the insulation. The drying of extract containing solvent (Petroleum ether, ethyl acetate, ethanol and distilled water) was done by vacuum distillation process. Percentage yield was shown in Table 2.

Table 2: Percentage yield of different extract

Parts	Solvents	Extract color	Yield (in gm)	% Yield w/w
FMC (fruits of *Momordica charantia*)	PFMC	Yellowish green	5.54	6.93
	EFMC	Brown	4.72	5.9
	AFMC	Dark Brown	16.05	20.06
	QFMC	Greenish brown	13.08	16.35
LPG (leaves *of Pongamia glabra*)	PLPG	Greenish brown	1.624	2.03
	ELPG	Brown	2.94	3.675
	ALPG	Dark Brown	6.264	7.83
	QLPG	Brownish Black	5.024	6.28
FPN (fruits of *Piper nigrum*)	PFPN	Yellowish green	2.304	2.88
	EFPN	Brown	1.664	2.08
	AFPN	Dark Brown	10.158	12.698
	QFPN	Greenish brown	8.904	11.13

Where PFMC- Petroleum ether extract of *Momordica charantia* fruits**, EFMC-**Ethyl acetate extract of *Momordica charantia* fruits**, AFMC-** Etahnolic extract of *Momordica charantia* fruits**, QFMC-** Aqueous extract of *Momordica charantia* fruits, **PLPG-** Petroleum ether extract of *Pongamia glabra* leaves**, ELPG-**Ethyl acetate extract of *Pongamia glabra* leaves**, ALPG-** Etahnolic extract of *Pongamia glabra* leaves**, QLPG-** Aqueous extract of *Pongamia glabra* leaves, **PFPN-** Petroleum ether extract of *Piper nigrum* fruits**, EFPN-**Ethyl acetate extract of *Piper nigrum* fruits**, AFPN-** Etahnolic extract of *Piper nigrum* fruits**, QFPN-** Aqueous extract of *Piper nigrum* fruits.

Phytochemical Screening

Preliminary Phytochemical screening was performed for extracts of *Momordica charantia* fruits (FMC), *Pongamia glabra* (LPG) and *Piper nigrum* fruits (FPN). It was noted that extracts of FMC contain flavonoids, glycosides, alkaloids, tannins, carbohydrates, saponins, steroids, fats, oils, protein and amino acids. The petroleum ether extract PFMC contains fats and fixed oils. The ethyl acetate extract EFMC had shown the presence of glycosides and steroids. The alcoholic extract AFMC had given the positive results with alkaloids, tannins, carbohydrates, glycosides, flavonoids, proteins and amino acids. The aqueous extract QFMC has shown the presence of alkaloids, tannins, carbohydrates, saponins, flavonoid s, proteins and amino acids. The flavonoid al content test for qualitative analysis was given with large intensity for alcoholic and aqueous extracts of the fruits of M*omordica charantia.* Result of phytochemical screening of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* was show in Table 3.1, 3.2 and 3.3 simultaneously.

Table 3.1: Qualitative analysis of different Extract of *Momordica charantia* fruits

S. No	Test	PFMC	EFMC	AFMC	QFMC
1.	**ALKALOIDS**				
a.	Dragendorff's test	-ve	-ve	+ve	+ve
b.	Hager's test	-ve	-ve	+ve	+ve
c.	Wagner's test	-ve	-ve	+ve	+ve
d.	Mayer's test	-ve	-ve	+ve	+ve
2.	**TANNINS**				
a.	Vanillin-HCl test	-ve	-ve	+ve	+ve
b.	Ferric chloride test	-ve	-ve	+ve	+ve
c.	Gelatin test	-ve	-ve	+ve	+ve
3.	**CARBOHYDRATES**				
a.	Molish test	-ve	-ve	+ve	+ve
b.	Fehling test	-ve	-ve	+ve	+ve
b.	Beneedicts test	-ve	-ve	+ve	+ve
4.	**GLYCOSIDE**				
a.	Keller Killani	-ve	+ve	+ve	-ve
b.	Legal test	-ve	+ve	+ve	-ve
c.	Borntrager test	-ve	+ve	+ve	-ve
5.	**SAPONINS**				
a.	Foam test	-ve	-ve	-ve	+ve
6.	**FLAVONOIDS**				
a.	Shinoda test	-ve	-ve	++ve	++ve
b.	Lead Acetate test	-ve	-ve	++ve	+ve
7.	**STEROIDS**				
a.	LibermannBurchard test	-ve	+ve	-ve	-ve
b.	Salkowski Reaction	-ve	+ve	-ve	-ve
8.	**FATS & OILS**				
a.	Filter paper Test	+ve	-ve	-ve	-ve
b.	Dye Test	+ve	-ve	-ve	-ve
9.	**PROTEINS AND AMINO ACIDS**				
a.	Millions Test	-ve	-ve	+ve	+ve
b.	Biuret Test	-ve	-ve	+ve	+ve
c.	Precipitation Test	-ve	-ve	+ve	+ve
d.	Ninhydrin Test	-ve	-ve	+ve	+ve

Table 3.2: Qualitative analysis of different Extract of *Pongamia glabra* leaves

S. No	Test	PLPG	ELPG	ALPG	QLPG
1	**ALKALOIDS**				
a.	Dragendorff's test	-ve	+ve	++ve	++ve
b.	Hager's test	-ve	+ve	+ve	+ve
c.	Wagner's test	-ve	+ve	+ve	+ve
d.	Mayer's test	-ve	+ve	+ve	+ve
2	**TANNINS**				
a.	Vanillin-HCl test	-ve	-ve	+ve	+ve
b.	Ferric chloride test	-ve	-ve	+ve	+ve
c.	Gelatin test	-ve	-ve	+ve	+ve
3	**CARBOHYDRATES**				
a.	Molish test	-ve	-ve	+ve	++ve
b.	Fehling test	-ve	-ve	+ve	++ve
b.	Beneedicts test	-ve	-ve	+ve	+ve
4	**GLYCOSIDE**				
a.	Keller Killani	-ve	-ve	++ve	-ve
b.	Legal test	-ve	-ve	+ve	-ve
c.	Borntrager test	-ve	-ve	+++ve	-ve
5	**SAPONINS**				
a.	Foam test	-ve	-ve	-ve	+ve
6	**FLAVONOIDS**				
a.	Shinoda test	-ve	-ve	++ve	++ve
b.	Lead Acetate test	-ve	-ve	++ve	+ve
7	**STEROIDS**				
a.	LibermannBurchard test	+ve	-ve	-ve	-ve
b.	Salkowski Reaction	+ve	-ve	-ve	-ve
8	**FATS & OILS**				
a.	Filter paper Test	++ve	-ve	-ve	-ve
b.	Dye Test	+ve	-ve	-ve	-ve
9	**PROTEINS AND AMINO ACIDS**				
a.	Millions Test	-ve	-ve	+ve	+ve
b.	Biuret Test	-ve	-ve	+ve	+ve
c.	Precipitation Test	-ve	-ve	-ve	-ve
d.	Ninhydrin Test	-ve	-ve	+ve	+ve

The extracts of LPG contain flavonoids, glycosides alkaloids, tannins, carbohydrates, saponins, steroids, fats, oils, protein and amino acids. The petroleum ether extract PLPG contains fats, fixed oils and steroids. The ethyl acetate extract ELPG had shown the presence of alkaloids. The alcoholic extract ALPG had given the positive results with alkaloids, tannins, carbohydrates, glycosides, flavonoids, proteins and amino acids. The aqueous extract QLPG has shown the presence of alkaloids, tannins, carbohydrates, saponins, flavonoid s, proteins and amino acids. The extracts of FPN contain alkaloids, flavonoid s, tannins, carbohydrates, glycosides, steroids, fats, oils, protein and amino acids. The petroleum ether extract PFPN contains fats, volatile oil and fixed oils. The ethyl acetate extract EFPN had shown the presence of alkaloids, steroids, fats and oils. The alcoholic extract AFPN had given the positive results with alkaloids, tannins, glycosides, flavonoid s, proteins and amino acids. The aqueous extract QFPN has shown the presence of alkaloids, tannins, carbohydrates, flavonoids, proteins and amino acids.

Table 3.3: Qualitative analysis of different Extract of *Piper nigrum* fruits

S.no	Test	PFPN	EFPN	AFPN	QFPN
1	**ALKALOIDS**				
a.	Dragendorff's test	-ve	+ve	+++ve	++ve
b.	Hager's test	-ve	+ve	+++ve	+++ve
c.	Wagner's test	-ve	+ve	++ve	+ve
d.	Mayer's test	-ve	+ve	+++ve	++ve
2	**TANNINS**				
a.	Vanillin-HCl test	-ve	-ve	+ve	+ve
b.	Ferric chloride test	-ve	-ve	+ve	+ve
c.	Gelatin test	-ve	-ve	+ve	+ve
3	**CARBOHYDRATES**				
a.	Molish test	-ve	-ve	-ve	+ve
b.	Fehling test	-ve	-ve	-ve	+ve
b.	Beneedicts test	-ve	-ve	-ve	+ve
4	**GLYCOSIDE**				
a.	Keller Killani	-ve	-ve	+ve	-ve
b.	Legal test	-ve	-ve	+ve	-ve
c.	Borntrager test	-ve	-ve	+ve	-ve
5	**SAPONINS**				
a.	Foam test	-ve	-ve	-ve	-ve
6	**FLAVONOIDS**				
a.	Shinoda test	-ve	+ve	++ve	++ve
b.	Lead Acetate test	-ve	+ve	++ve	+ve
7	**STEROIDS**				
a.	LibermannBurchard test	-ve	+ve	-ve	-ve
b.	Salkowski Reaction	-ve	+ve	-ve	-ve
8	**FATS & OILS**				
a.	Filter paper Test	+ve	+ve	-ve	-ve
b.	Dye Test	++ve	+ve	-ve	-ve
9	**PROTEINS AND AMINO ACIDS**				
a.	Millions Test	-ve	-ve	+ve	+ve
b.	Biuret Test	-ve	-ve	+ve	+ve
c.	Precipitation Test	-ve	-ve	-ve	-ve
d.	Ninhydrin Test	-ve	-ve	++ve	++ve

Where PFPN- Petroleum ether Extract of *Piper nigrum*fruits, **EFPN-**Ethyl acetateExtract of *Piper nigrum*fruits, **AFPN-** Etahnolic Extract of *Piper nigrum*fruits, **QFPN-** Aqueous Extract of *Piper nigrum*fruits. '-' means negative result, '+' means positive results, '++' or '+++' means intensity of result

Quantitative Analysis

Quantitative analysis was performed to determine the total phenolic compounds.

The concentration of phenolics in various plant extracts was determined using spectrophotometric method with Folin-Ciocalteu reagent. The content of phenolics was expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.044x-0.022$, $r^2 = 0.997$) (Figure 4), mg of QE/g of extract **(Table 4, 5 & 6)**.

Determination of Total Phenolic Content

Table 4: Standard Curve of Gallic Acid

Concentration (µg/ml)	Absorbance (Mean)
0.8	0.0415±0.051
1.6	0.0841±0.043
3.12	0.1589±0.047
6.25	0.3178±0.028
12.5	0.6081±0.034
25	1.119±0.062

The aqueous extract of fruits of *Momordica charantia* (QMC) has shown highest amount of total phenolic content (64.091mgGAE/g) as compared to others. The aqueous extract of fruits has more phenolic content than the other extracts of *momordica*. E.g. QMC> AMC> EMC> PMC. It contains 40.50%, 12.05% and 3.59% more flavonoid s content than PMC, EMC and AMC respectively.

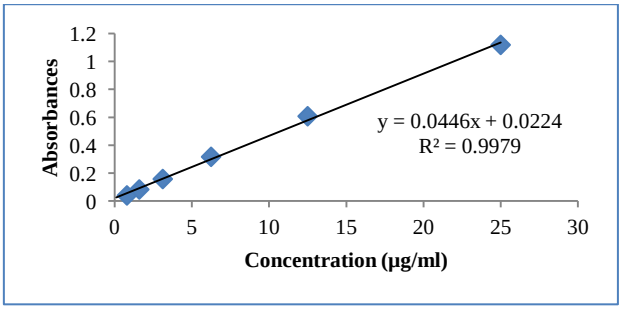

Figure 4: Standard Curve of Gallic Acid

Different concentrations (200, 500 and 1000 µg/ml) has been taken to calculate the total phenolic content in different extracts obtained from *Momordica charantia*. Concentration dependent phenolic content variation has

validated the analytical methodology e.g. spectroscopy. The total phenolic content was significantly more in the aqueous extract of *Momordica charantia* (56.88 or 56.90µg/ml) for 1000µg/ml, when compared to other concentrations (36.40 and 12.50µg/ml) 500 and 200 µg/ml respectively **(Table 5).** The concentration of phenolics was also observed more in polar solvents. It was increasing with increase in polarity e.g. petroleum ether< ethyl acetate< alcoholic< aqueous. Almost observed in extracts obtained after extraction. Summary conclude that the total phenolic content in fruits of *Momordica charantia* will be (PMC+EMC+AMC+QMC) 220.38mgGAE/g.

The alcoholic extract of leaves of *Pongamia glabra* (QPG) has shown highest amount of total phenolic content (59.386 mgGAE/g) as compared to others. The alcoholic extract of leaves has more phenolic content than the other extracts of *pongamia*. Eg. APG> QPG> EPG> PPG It contains 41.54%, 7.03% and 1.51% more flavonoid s content than PPG, EPG and QPG respectively.

Different concentrations (200, 500 and 1000 µg/ml) has been taken to calculate the total phenolic content in different extracts obtained from *Pongamia glabra*. Concentration dependent phenolic content variation has validated the analytical methodology e.g. spectroscopy. The total phenolic content was significantly more in the alcoholic extract of *Pongamia glabra* (51.70µg/ml) when compared to other concentrations (33.60 and 11.30µg/ml) 500 and 200 µg/ml respectively. The concentration of phenolics was also observed more in polar solvents. It was increasing with increase in polarity e.g. petroleum ether< ethyl acetate< alcoholic< aqueous, almost observed in extracts obtained after extraction, but alcoholic extract has shown more phenolic content than aqueous. Summative the total phenolic content in leaves of *Pongamia glabra* will be (PPG+EPG+APG+QPG) 207.78mgGAE/g. The alcoholic extract of fruits of *Piper nigrum* has shown highest amount of total phenolic content (57.76mgGAE/g) as compared to others. The alcoholic extract of fruits has more phenolic content than the other extracts of *piper*. Eg. APN> QPN> EPN> PPN

Table 5: Total Phenolic Content in various concentrations of different extracts

S. No.		1				2				3			
Drugs		FMC				LPG				FPN			
Conc(µg /ml)	Extracts	PMC	EMC	AMC	QMC	PPG	EPG	APG	QPG	PPN	EPN	APN	QPN
200	Abs.	0.31	0.47	0.51	0.53	0.28	0.46	0.49	0.47	0.42	0.46	0.47	0.46
		0.31	0.46	0.52	0.53	0.29	0.45	0.48	0.48	0.42	0.45	0.47	0.46
	Conc(µg/ml)	7.57	11.11	12.14	12.50	6.82	10.86	11.61	11.23	9.98	11.00	11.16	11.05
		7.43	10.84	12.36	12.57	7.07	10.80	11.45	11.36	10.09	10.80	11.18	10.93
	Avg Conc	7.50	10.98	12.25	12.53	6.94	10.83	11.53	11.30	10.03	10.90	11.17	10.99
500	Abs.	0.92	1.40	1.52	1.58	0.83	1.36	1.49	1.47	1.25	1.38	1.44	1.42
		0.93	1.35	1.53	1.57	0.84	1.34	1.47	1.45	1.24	1.29	1.42	1.41
	Conc.(µg/ml)	21.43	32.30	34.95	36.45	19.45	31.32	34.32	33.84	28.86	31.95	33.18	32.75
		21.68	31.09	35.36	36.27	19.61	31.00	33.95	33.43	28.70	29.89	32.82	32.55
	Avg. Conc	21.56	31.69	35.16	36.36	19.53	31.16	34.14	33.64	28.78	30.92	33.00	32.65
1000	Abs.	1.45	2.21	2.34	2.49	1.32	2.14	2.26	2.25	1.97	2.20	2.24	2.23
		1.48	2.22	2.35	2.47	1.31	2.14	2.30	2.26	1.97	2.19	2.24	2.22
	Conc.(µg/ml)	33.55	50.75	53.70	57.05	30.45	49.23	51.77	51.57	45.34	50.39	51.36	51.16
		34.02	50.91	53.89	56.70	30.25	49.07	52.66	51.82	45.16	50.20	51.50	51.05
	Avg. Conc	33.78	50.83	53.80	56.88	30.35	49.15	52.22	51.69	45.25	50.30	51.43	51.10

It contains 11.71%, 3.84% and 1.11% more phenolic content than PPN, EPN and QPN respectively. Different concentrations (200, 500 and 1000 µg/ml) has been taken to calculate the total phenolic content in different extracts obtained from *Piper nigrum*. Concentration dependent phenolic content variation has validated the analytical methodology e.g. spectroscopy. The total phenolic content was significantly more in the alcoholic extract of *Piper nigrum*(51.4µg/ml) when compared to other concentrations (33.0 and 11.20µg/ml) 500 and 200 µg/ml respectively.

The concentration of phenolic was also observed more in polar solvents. It was increasing with increase in polarity e.g. petroleum ether< ethyl acetate< alcoholic< aqueous. Almost observed in extracts obtained after extraction, but alcoholic extract has shown more phenolic content than aqueous in the case of *Piper nigrum*. Summative the total phenolic content in leaves of *Piper nigrum* will be (PPN+EPN+APN+QPN) 221.41mgGAE/g. The total phenolic content of *Piper nigrum* (PN) fruits was found to be largest among the other drugs e.g. *Pongamia glabra* (PG) and *Momordica charantia* (MC). PN>MC> PG (221.41>220.38>207.78)

The fruits have more phenolic content in the given samples when compared with the leaves. It is also evident from the obtained results that the alcoholic and aqueous extracts have more phenolic content than the other extracts of the same drugs e.g. (PMC+EMC) [94.5mgGAE/g] < (AMC+QMC) [125.88mgGAE/g], (PPG+EPG) [89.91mgGAE/g] < (APG+QPG) [117.87mgGAE/g] and (PPN+EPN) [106.53mgGAE/g] < (APN+QPN) [114.88mgGAE/g]. The Hydroalcoholic extract may contain more phenolic content than the individual solvent extract. The combined total phenolic content in the alcoholic and aqueous extracts of the drugs have following observations: AMC+QMC> APG+QPG> APN+QPN [125.87mgGAE/g> 117.86mgGAE/g > 114.88mgGAE/g respectively]

The total phenolic content of all petroleum ether extracts of various drugs was found very less in quantity. Only the *piper nigrum* fruit extract has more amount amongst the other extracts e.g. 50.99mgGAE/g. (Table 8). The total phenolic content in different plant extracts found can be represented in following manner: QMC> AMC> APG> QPG> APN> QPN> EMC> EPN> EPG> PPN> PMC> PPG. The concentration of total phenols in different plant extracts

was found in following ascending order:PPG< PMC< PPN< EPG< EPN< EMC< QPN< APN< QPG< APG< AMC< QMC.

Table 7: Gallic Acid Equivalent In mg/g Extract

Extracts	200 (µg/ml)	Qty 1	500 (µg/ml)	Qty 2	1000 (µg/ml)	Qty 3	Avg. mgGAE/g
PMC	7.50	37.50	21.56	43.11	33.78	33.78	38.13
EMC	10.98	54.89	31.69	63.39	50.83	50.83	56.37
AMC	12.25	61.25	35.16	70.32	53.80	53.80	61.79
QMC	12.53	62.67	36.36	72.73	56.88	56.88	64.09
PPG	6.94	34.72	19.53	39.07	30.35	30.35	34.71
EPG	10.83	54.15	31.16	62.32	49.15	49.15	55.20
APG	11.53	57.67	34.14	68.27	52.22	52.22	59.39
QPG	11.30	56.48	33.64	67.27	51.69	51.69	58.48
PPN	10.03	50.17	28.78	57.57	45.25	45.25	51.00
EPN	10.90	54.49	30.92	61.84	50.30	50.30	55.54
APN	11.17	55.85	33.00	66.00	51.43	51.43	57.76
QPN	10.99	54.94	32.65	65.30	51.10	51.10	57.11

Table 8: Total Phenolic Content in Different Extracts

Extracts	Conc. mgGAE/g
PMC	38.133
EMC	56.367
AMC	61.788
QMC	64.091
PPG	34.712
EPG	55.205
APG	59.386
QPG	58.481
PPN	50.996
EPN	55.542
APN	57.761
QPN	57.114

Determination of Total Flavonoid Content

The concentration of flavonoids in various plant extracts was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: y = 0.001x-0.002, r2 = 0.998), mg of QE/g of extract **(Table 9, 10 and 11)**. Standard Curve of Quercetin was depicted in **Figure 5.** The concentration of flavonoids in different plant extracts was found in following ascending order:

PPG< PMC< PPN< EPG< EPN< EMC< QPN< APN< QPG< APG< AMC< QMC

Table 9: Standard Curve of Quercetin

Concentration (µg/ml)	Absorbance (Mean)
10	0.0089±0.0020
20	0.0172±0.0030
30	0.0252±0.0034
40	0.0348±0.0029
50	0.0438±0.0024

n=3

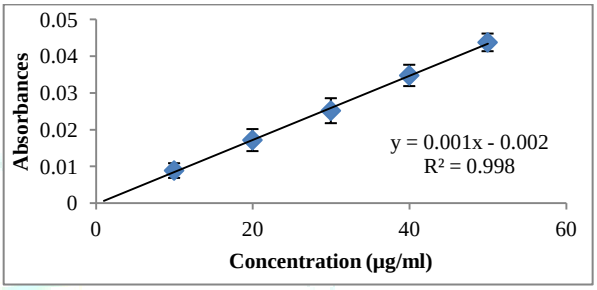

Figure 5: Standard Curve of Quercetin

The aqueous extract of fruits of *Momordica charantia* (QMC) has shown highest amount of total flavonoid content (842.27mgQE/g) as compared to others. The aqueous extract of fruits has more flavonoid content than the other extracts of *momordica charantia*. E.g. QMC> AMC> EMC> PMC. It contains 52.64%, 30.78% and 11.43% more flavonoids content than PMC, EMC and AMC respectively. Different concentrations (200, 500 and 1000 µg/ml) has been taken to calculate the total flavonoid content in different extracts obtained from *Momordica charantia*. Concentration dependent flavonoid content variation has validated the analytical methodology e.g. spectroscopy. The total flavonoid content was significantly more in the aqueous extract of *Momordica charantia* (808.64 µg/ml) when compared to other concentrations (411.36µg/ml and 179.09µg/ml) 500 and 200 µg/ml respectively **(Table 10).** Summative the total flavonoids content in fruits of *Momordica charantia* will be 2570.20 mgQE/g.

The alcoholic extract of leaves of *Pongamia glabra* (APQ) has shown highest amount of total flavonoid content (721.97 mgQE/g) as compared to others. The alcoholic extract of leaves has more flavonoid content than the other extracts of *Pongamia glabra*. E.g. APG> QPG> EPG> PPG. It contains 51.23%, 30.39% and 7.64% more flavonoids content than PPG, EPG and QPG respectively. Different concentrations (200, 500 and 1000 µg/ml) has been taken to calculate the total flavonoid content in different extracts obtained from *Pongamia glabra*. Concentration dependent flavonoid content variation has validated the analytical methodology e.g. spectroscopy. The total flavonoid content was significantly more in the alcoholic extract of *Pongamia glabra* (685.46µg/ml) when compared to other concentrations (302.73µg/ml and 175.00µg/ml) 500 and 200 µg respectively **(Table 10).** Summative the total flavonoids content in leaves of *Pongamia glabra* will be (PPG+EPG+APG+QPG) 2243.50 mgQE/g.

Table 10: Total Flavonoid Content in various concentrations of different extracts

S. No.		1				2				3			
Drugs		FMC				LPG				FPN			
Conc.	Extracts	PMC	EMC	AMC	QMC	PPG	EPG	APG	QPG	PPN	EPN	APN	QPN
200 µg/ml	Abs.	0.10	0.14	0.20	0.18	0.08	0.12	0.19	0.18	0.11	0.13	0.17	0.16
		0.09	0.15	0.21	0.21	0.08	0.11	0.20	0.15	0.10	0.14	0.17	0.16
	Conc. (µg/ml)	87.27	127.27	181.09	166.36	70.00	107.27	171.82	160.91	99.09	116.36	153.64	141.82
		81.82	135.45	187.36	191.82	72.73	100.00	178.18	140.91	89.09	123.64	157.27	147.27
	Avg. Conc.	84.55	131.36	184.23	179.09	71.36	103.64	175.00	150.91	94.09	120.00	155.45	144.55
500 µg/ml	Abs.	0.21	0.31	0.33	0.44	0.20	0.30	0.33	0.35	0.27	0.31	0.34	0.33
		0.23	0.32	0.35	0.47	0.19	0.28	0.34	0.36	0.26	0.30	0.35	0.32
	Conc. (µg/ml)	187.27	285.45	301.82	396.36	179.09	270.91	300.00	316.36	243.64	280.00	310.91	298.18
		211.82	290.91	314.55	426.36	171.82	257.27	305.45	326.36	239.09	275.45	317.27	292.73
	Avg. Conc.	199.55	288.18	308.18	411.36	175.45	264.09	302.73	321.36	241.36	277.73	314.09	295.45
1000 µg/ml	Abs.	0.41	0.57	0.76	0.88	0.39	0.50	0.74	0.68	0.45	0.55	0.65	0.64
		0.41	0.56	0.78	0.90	0.38	0.52	0.77	0.64	0.45	0.56	0.65	0.65
	Conc. (µg/ml)	377.27	520.00	693.64	801.82	351.82	450.91	674.55	620.91	409.09	498.18	592.73	584.55
		372.73	511.82	707.27	815.45	345.45	471.82	696.36	585.45	413.64	505.45	590.00	589.09
	Avg. Conc.	375.00	515.91	700.45	808.64	348.64	461.36	685.45	603.18	411.36	501.82	591.36	586.82

The alcoholic extract of fruits of *Piper nigrum* has shown highest amount of total flavonoid content (665.61 mgQE/g) as compared to others. The alcoholic extract of fruits has more flavonoid content than the other extracts of *piper*. E.g. APN> QPN> EPN> PPN. It contains 31.66%, 17.01% and 4.83% more flavonoids content than PPN, EPN and QPN respectively. Different concentrations (200, 500 and 1000 µg/ml) has been taken to calculate the total flavonoid content in different extracts obtained from *Piper nigrum*. Concentration dependent flavonoid content variation has validated the analytical methodology e.g. spectroscopy. The total flavonoid content was significantly more in the alcoholic extract of *Piper nigrum* (591.36µg/ml) when compared to other concentrations (314.09µg/ml and 155.46µg/ml) 500 and 200 µg/ml respectively **(Table 10).** Summative the total flavonoids content in leaves of *Piper nigrum* will be (PPN+EPN+APN+QPN) 2306.40 mgQE/g.

Table 11: Quercetin equivalent in mg/g extract

Extracts	200 (µg/ml)	Qty.1	500 (µg/ml)	Qty.2	1000 (µg/ml)	Qty.3	Avg. Qty. (mgQE/g)
PMC	84.55	422.73	199.55	399.09	375.00	375.00	398.94
EMC	131.36	656.82	288.18	576.36	515.91	515.91	583.03
AMC	184.23	921.14	308.18	616.36	700.45	700.45	745.98
QMC	179.09	895.45	411.36	822.73	808.64	808.64	842.27
PPG	71.36	356.82	175.45	350.91	348.64	348.64	352.12
EPG	103.64	518.18	264.09	528.18	461.36	461.36	502.58
APG	175.00	875.00	302.73	605.45	685.45	685.45	721.97
QPG	150.91	754.55	321.36	642.73	603.18	603.18	666.82
PPN	94.09	470.45	241.36	482.73	411.36	411.36	454.85
EPN	120.00	600.00	277.73	555.45	501.82	501.82	552.42
APN	155.45	777.27	314.09	628.18	591.36	591.36	665.61
QPN	144.55	722.73	295.45	590.91	586.82	586.82	633.48

The total flavonoids content of *Momordica charantia* fruits was found to be largest among the other drugs e.g. *Pongamia glabra* and *Piper nigrum.* MC> PN> PG (2570.20> 2306.4> 2243.50). The fruits have more flavonoids content in the given samples when compared with the leaves. It is also evident from the obtained results that the alcoholic and aqueous extracts have more flavonoids content than the other extracts of the same drugs e.g. (PMC+EMC) [981.97 mgQE/g]< (AMC+QMC) [1588.26 mgQE/g], (PPG+EPG) [854.70 mgQE/g]< (APG+QPG) [1388.79 mgQE/g] and (PPN+EPN) [1007.27 mgQE/g]< (APN+QPN) [1299.09 mgQE/g]. The Hydroalcoholic extract may contain more flavonoids content than the individual solvent extract. The combined total flavonoids content in the alcoholic and aqueous extracts of the drugs have following observations:

AMC+QMC> APG+QPG> APN+QPN [1588.26> 1388.79> 1299.09 mgQE/g respectively]. The total flavonoids content all petroleum ether extracts of various drugs was found very less in quantity. Only the *piper nigrum* fruit extract has more amount amongst the other extracts e.g 454.85 mgQE/g. The total flavonoids content in different plant extracts found can be represented in following manner: QMC> AMC> APG> QPG> APN> QPN> EMC> EPN> EPG> PPN> PMC> PPG.

Conflicts of interest

The author declares no conflicts of interest.

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