



RESEARCH ARTICLE

QUANTITATIVE DETERMINATION OF SOME PHYTOCHEMICAL CONTENTS OF VARIOUS PLANT SEEDS AND LEAVES OF WATER, ETHANOL AND METHANOL EXTRACTS

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ABSTRACT

The present study found out percent composition of Alkaloids, Flavonoids, Saponins and Terpenoids from the leaves and seeds of *Luffa acutangula*, *Luffa aegyptiaca*, *Cucurbita moschata* and *Momordica charantia*. The water, ethanol and methanol extracts were prepared and the percentage of above contents were determined in different extracts. The RF values of all contents for different extracts of leaves and seeds were determined by Thin Layer Chromatography using various spraying agents.

INTRODUCTION

L. acutangula, *L.aegyptiaca*, *C.moschata* and *M. charantia* are main plants in Vidarbha region. These plants generally active in rainy season. The fruits of the plants are one of the basic vegetable for peoples.

***Luffa acutangula*:** The fruits of *L.acutangula* used in diabetes, immune modulation, tumor suppression, parkinsonism, as an antimicrobial, in ulcers and for hepatoprotection (Vaidya, 2016). *L. acutangula* is a pan tropical climbing herb and cultivated throughout India and can grow in all types of soils (Manikandaselvi, 2016). It commonly called as Ranturai, Kadudodak in Marathi.

***Momordica charantia*:** *M. charantia* belongs to family Cucurbitaceae is the medicinal plants with hypoglycemic activity being studied extensively. It is a climber widely cultivated as food in Asia, Africa and South America (Desai, 2015). It contain non-nitrogenous neutral principle charantin, and on hydrolysis gives glucose and a sterol (Sampath Kumar, 2010). It is commonly called as Bitter gourd which is one of the most popular vegetables in Southeast Asia (Kaur, 2014).

***Luffa aegyptiaca*:** The plant '*Luffa aegyptiaca*' commonly known as sponge gourd belongs to the Cucurbitaceae family. It is a vigorous climbing annual plant with several lobed cucumber-like leaves (Mankilik, 2014). Fruits of these plants are smooth, cylindrical and usually 20-30 cm long. Seeds are black or gray and are compressed (Nirmal, 2009).

***Cucurbita moschata*:** It is also called as Butternut squash or pumpkin which is vigorous growing plant that takes 85 to 90 days to maturity and matured fruit weigh about 500 to 1000gm (Isaboke, 2012). Pumpkin found in many shapes, sizes and colors and good source of carotene, pectin, mineral salts, vitamins and other substances that are beneficial to health. Weed control is a major problem for butternut squash production that need to be considered by growers. The total number of fruits per plant of *C. moschata* was highly correlated with the yield but the fruit size was not correlated with the number of fruits per plant (Grisales, 2015).

MATERIALS AND METHODS

The plant materials for the present work were collected from Malkapur and Motala tahsil area & authenticated. The seeds and leaves were dried in shade & then powdered, sieved & then used for analysis. The methods were carried out for Aqueous, Ethanol and Methanol extracts (Suryanti, 2015; O Lucy, 2012; Jassal, 2014; Sinha, 2015 and Madhu, 2012).

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Taxonomical Classification (Sharma, 2011; Ramanujam, 2014 and Mhya, 2014)

Description	<i>Momordica Charantia</i>	<i>Luffa acutangula</i>	<i>Luffa Aegyptiaca</i>	<i>Cucurbita moschata</i>
Kingdom	Plantae	Plantae	Plantae	Plantae
Order	Cucurbitales	Cucurbitales	Cucurbitales	Cucurbitales
Family	Cucurbitaceae	Cucurbitaceae	Cucurbitaceae	Cucurbitaceae
Genus	Momordica	Luffa	Luffa	Cucurbita
Species	M. Charantia	L. Acutangula	L. Aegyptiaca	C. Moschata
Binomial Name	<i>Momordica Charantia</i>	<i>Luffa acutangula</i>	<i>Luffa Aegyptiaca</i>	<i>Cucurbita moschata</i>
Common Names	Bitter Guard, Bitter Melon, Balsom Pear	Silky guard, Ridge guard, Angeled loof	Smooth Guard, Sponge Guard, Rag Guard	
Marathi	Karle	Dodake	Gilke	Ran bhopla

Qualitative Phytochemical Analysis

Alkaloids

Wagner's reagent: A small amount of extract was taken and treated with 3-5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate or colouration. Wagner's reagent was prepared by mixing 1.27g of iodine and 2g of potassium iodide in 100ml of water.

Flavonoids

Alkaline reagent test: To the 2ml of each extract, a few drops of 20% NaOH solution were added. The formation of yellow color which becomes colorless on addition of dilute HCl, confirmed the presence of flavonoids.

Terpenoids

Salkowki's test: To 1ml of chloroform, 2ml of extract was added followed by the addition of few drops of conc. H₂SO₄. The formation of reddish brown precipitate produced immediately confirmed the presence of terpenoids.

Saponins

Foam test: 2ml of extract was taken and added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirmed the presence of saponins.

Quantitative Phytochemical Estimations

Estimation of Alkaloids: 1gm of powder sample was weighed into 250ml beaker and 100ml of solvent was added and allow to stand for 4 hours. It was filtered and the extract was concentrated on water bath. Then concentrated NH₄OH was added drop wise to the extract until the precipitation was complete. The solution was allow to settle and precipitate was collected and washed with dilute NH₄OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids: 50ml solvent was used to extract 5 g of sample repeatedly at room temperature and filtered through What man filter paper No 42. The filtrate was evaporated to dryness in a crucible over a water bath until a constant weight was reached.

Estimation of Terpenoids: 5 gm of powder sample were soak in respective solvent for 24hrs and then filter.

The filtrate was extracted with petroleum ether for two-three times. The ether layer was collected and the extract was dried and treated as total terpenoids.

Estimation of Saponin: 50cm³ of solvent was added to 10g of the sample in a conical flask and heated at 55°C over a hot water bath for 4 hours with continuous stirring. The mixture was filtered and residue re-extracted with 200ml solvent. The combined extracts were reduced to 40ml over a water bath at 90°C. 20ml of diethyl ether was added to the concentrate in a 250ml separating funnel and shaken vigorously. The aqueous layer was recovered and purification process repeated. 60ml of n-butanol was added to the extract and then washed twice with 10ml of 5% aqueous NaCl. The solution was evaporated in a water bath. The sample was oven-dried to a constant weight.

Thin Layer Chromatography

TLC plates were prepared for different extracts using silica gel. The plates were placed in developing chamber containing mixture of solvents listed in Table no-4. The R_f Values of some selected constituents like alkaloids, flavonoids, terpenoids and saponin were determined for water and ethanolic extracts were reported in Table no-3.

RESULT AND DISCUSSION

Quantitative Phytochemical Analysis: The phytochemical analysis indicates the presence of alkaloids, flavonoids, terpenoids and saponin in different quantities. The percentage of alkaloid has been detected in the leaves and seeds of the plants was in the range of 6-49%. The percentage of total flavonoid in leaves was highest for L₂ of water extract and that of for seeds was maximum for S₂ of ethanol extract. Terpenoids content was found to be in the range of 9-0.2%, which was maximum for L₁ of water extract and for L₃ of methanol extract. Saponin is regarded as high molecular weight compound and used in the preparation of traditional medicines. The maximum quantity of saponin was observed for S₃ of water extract (10%) and minimum for L₂ and L₄ of methanol extract (1.6%) The percentage of each constituent was recorded in Table no -2

Table 1. Plants and their code

Sr.No	Plant	Leaves	Seed
1	<i>Luffa acutangula</i>	L ₁	S ₁
2	<i>Luffa aegyptiaca</i>	L ₂	S ₂
3	<i>Cucurbita moschata</i>	L ₃	S ₃
4	<i>Momordica charantia</i>	L ₄	S ₄

Table 2. Percentage of constituents in different extracts

Extract	Constituent	Leaves (%)				Seeds (%)			
		L ₁	L ₂	L ₃	L ₄	S ₁	S ₂	S ₃	S ₄
Water	Alkaloids	24	34	22	38	17	30	5.0	19.8
	Flavonoids	14	34	16	20	11	26	10	30
	Terpenoids	09	1.2	5.0	0.4	2.8	1.2	1.6	3.0
	Saponin	4.6	4.2	7.0	4.4	7.0	3.2	10	1.8
Ethanol	Alkaloids	24	20	20	32	41	46	49	18
	Flavonoids	07	8.0	5.0	22	18	38	16	12.4
	Terpenoids	0.4	0.4	0.8	1.6	6.2	6.0	3.0	1.0
	Saponin	2.6	6.2	2.0	5.2	4.2	3.2	4.6	3.6
Methanol	Alkaloids	32	18	24	36	8.0	7.0	6.0	18
	Flavonoids	4.0	2.0	6.0	2.0	2.0	10	10	10
	Terpenoids	0.6	0.7	0.2	0.7	1.2	3.2	1.2	1.8
	Saponin	2.4	1.6	3.0	1.6	4.4	2.8	5.0	2.6

Table 3. The Rf values of constituents in different extracts

Extract	Constituent	Leaves Rf Values				Seeds Rf Values			
		L ₁	L ₂	L ₃	L ₄	S ₁	S ₂	S ₃	S ₄
Water	Alkaloids	0.75	0.59	0.67	0.69	0.42	0.70	0.59	0.40
	Flavonoids	0.61	0.57	0.65	0.70	0.62	0.62	0.62	0.75
	Terpenoids	0.42	0.62	0.58	0.58	0.75	0.71	0.57	0.76
	Saponin	0.55	0.84	0.90	0.52	0.86	0.82	0.51	0.86
Ethanol	Alkaloids	0.68	0.67	0.71	0.62	0.57	0.49	0.55	0.58
	Flavonoids	0.91	0.75	0.88	0.80	0.77	0.87	0.85	0.72
	Terpenoids	0.80	0.53	0.85	0.63	0.67	0.55	0.69	0.52
	Saponin	0.77	0.69	0.79	0.76	0.55	0.72	0.58	0.84
Methanol	Alkaloids	0.60	0.75	0.70	0.77	0.62	0.59	0.67	0.76
	Flavonoids	0.70	0.72	0.81	0.72	0.78	0.65	0.95	0.75
	Terpenoids	0.90	0.76	0.80	0.86	0.90	0.92	0.86	0.89
	Saponin	0.80	0.85	0.69	0.80	0.81	0.72	0.77	0.94

Table 4. Solvent system and Spraying agents with color developed

Constituent	Solvent system	Spraying Agent	Color Developed
Alkaloids	NH ₄ OH:CH ₃ OH 3:17	Mayer's reagent	Yellowish
Flavonoids	CHCl ₃ :CH ₃ OH 18:2	Iodine vapors	Reddish
Terpenoids	C ₆ H ₆ :CH ₃ COOC ₂ H ₅ 1:1	10% HS ₂ O ₄	Greenish
Saponin	CHCl ₃ :CH ₃ COOH: CH ₃ OH:H ₂ O 6:2:1:1	Iodine vapors	Brown

Thin layer Chromatography: The RF values for leaves and seeds of water, ethanol and methanol were determined and represented in Table-3 and solvent system and spraying agents are represented in Table-4. The highest RF value recorded for saponin of S₄ for methanol extract (0.94) and least value recorded for alkaloid of S₄ for water extract (0.40).

Conclusion

The present investigation attempted to evaluate quantitative phytochemical estimation of crude extracts from leaves and seeds of *L. acutangula*, *L. aegyptiaca*, *C. moschata* and *M. charantia*. Alkaloid contents was found to be highest for seeds extracts of water, ethanol and methanol as compare to leaves. The flavonoid content was maximum for water extract of L₂(34%) and minimum for methanol extract of L₂, L₄ and S₁(2%). Terpenoid contents was not exceeds 9% for any extract. The highest percentage of saponin was estimated for water extract of S₃(10%) and lowest percentage for methanol extract of L₂ and L₄(1.6%). Alkaloid is a class of nitrogen containing natural compound. Quite a high percentage of alkaloid has been detected in the leaves and seeds of the

selected plants. The phytochemical characterization of the extracts, the identification and detection of responsible bioactive compounds and quality standards are necessary for future study.

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