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IN VITRO QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOLIC AND 50% ETHANOLIC EXTRACTS OF *TINOSPORA CORDIFOLIA*, *MOMORDICA CHARANTIA*, *CUCURBITA MAXIMA* AND *RAPHANUS SATIVUS*

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ABSTRACT: Phytochemicals are non-nutritive chemical constituents of plants which occur naturally in it such as Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. The aim of the present study was to carryout qualitative and quantitative phytochemical analysis of ethanolic and 50% ethanolic extracts of *Tinospora cordifolia* (stems), *Momordica chirantia* (fruit), *Cucurbita maxima* (fruit) and *Raphanus sativus* (root). The result of the qualitative phytochemical screening revealed the presence of various secondary metabolites with highest activity in 50% ethanolic extracts of *T. cordifolia*, *C. maxima* and ethanolic extract of *Tinospora cordifolia*. Quantitative phytochemical analysis revealed that the total flavonoids content was found to be varied in different extracts wherein high activity was noted in 50% ethanolic extract of *T.cordifolia* (9.91 ± 1.46 mg CE/gm) followed by 50 % ethanolic extract of *Cucurbita maxima* (9.79 ± 0.35 mg CE/gm) and absolute ethanolic extract of *T.cordifolia* (9.75 ± 0.4 mg CE/gm) among all 8 tested extracts. Total phenol content was significantly high in 50% ethanolic extracts of *Tinospora cordifolia* (24.70 ± 2.04 mg GAE/gm) followed by 50% ethanolic extract of *Cucurbita maxima* (18.23 ± 0.03 GAE/gm) and absolute ethanolic extract of *Tinospora cordifolia* (12.33 ± 0.5 mg GAE/gm).

INTRODUCTION: Phytochemicals are bioactive substances of plants that have been associated in the protection of human and animal health against chronic degenerative diseases. The major groups of phytochemicals that may contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids and the traditional antioxidant vitamins such as vitamin C and vitamin E. *Tinospora cordifolia* (Hindi name–Guduchi) is a climbing deciduous shrub.

It is found throughout tropical part of India and also found in China, Bangladesh, Myanmar & Srilanka. This plant belongs to the family Menispermaceae¹. Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties²⁻³. *Momordica charantia* (Karela) commonly known as bitter gourd, bitter melon or balsam pear is an economically important medicinal plant belonging to the family Cucurbitaceae⁴. The local traditional practitioners use the fruit of *Momordica Charantia* as a medicine for a variety of disorders. *Momordica Charantia* is a well-known to possess antihyperglycemia, anticholesterol, immuno-



suppressive, antiulcerogenic, anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, anti-microbial, anticholesterol, immunosuppressive, and anti-tumor activities⁵. *Cucurbita Maxima* (Pumpkin) belongs to the genus of *Cucurbita* and the family Cucurbitaceae and it is monoecious. *R. sativus niger* belongs to family Brassicaceae (order - Brassicales, subspecies-niger and variety-niger). It is a food crop, mostly an ingredient of salads in Asian countries during winter. Aim of the present study was to determine phytochemical compounds qualitatively and quantitatively, by *in vitro* testing in various vegetable/plant extracts.

MATERIALS AND METHODS:

Plant materials: Following plant materials viz., *Tinospora cordifolia* (stem), *Momordica charantia* (fruit), *Cucurbita maxima* (fruit), *Raphanus Sativus* (root) were collected in and around Bareilly (U.P) area and air dried and grinded to powder form and were subjected to extraction by absolute ethanolic and 50% ethanolic by columnar soxhlet method at temperature of 40-41°C with standard protocol. The extracts were dried at 41°C temperature.

In vitro qualitative phytochemical tests:

- Dragendroff's Test:** Extracts were dissolved individually in N/10 Hydrochloric acid and filtered. 1-2 ml filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids⁶.
- FeCl₃ Test:** Extracts were dissolved individually in N/10 Hydrochloric acid and filtered. 1-2 ml filtrates were treated with 5% FeCl₃. Appearance of greenish black ring indicated the presence of phenols⁶.
- Foam Test:** 0.5 gm of extract was shaken with 2 ml of water. If foam produced persisted for ten minutes it indicated the presence of saponins⁶.
- Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins⁶.

In vitro quantitative phytochemical tests:

- Total Flavonoids Content (TFC):** The total flavonoids content of the extracts and the potencies were determined according to colorimetric method⁷. In brief, the sample solution (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% NaNO₂ solution (0.15 ml). After 6 min of incubation, 10% AlCl₃ solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 4% NaOH solution (2 ml) to the mixture. Consequently, water was added to the sample to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm. The total flavonoid content was expressed in mg of catechin equivalent (CE) per gram of extract.
- Total Phenol Content (TPC):** The total phenolic content in the extracts and the potencies was measured using Folin-Ciocalteu reagent method⁸. The samples (0.4 mL) (1mg/mL extracts) were transferred into test tubes. To this solution, distilled water (1.0 mL) and Folin-Ciocalteu reagent (1.0 mL) were added, and the tubes shaken thoroughly. After 1 min, sodium carbonate solution (Na₂CO₃, 1.6 mL, 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. A linear dose response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm using UV spectrophotometer. The total phenolic compounds concentration in the extract and potencies was expressed as milligrams of gallic acid equivalent per gram of dry weight of extract (mg GAE/g).

RESULTS: The presences of different phytochemical compounds in 8 tested plant extracts are shown in **Table 1 and Figure 1**. Highly positive (++) alkaloids were noticed in 50 % ethanolic extracts of *T.cordifolia*, *C.maxima* (50% ethanolic) and ethanolic extract of *R.Sativus*. Ethanolic and 50% ethanolic extract of *M.charantia* showed positive (+) alkaloid reaction by Dragendroff's method.

Highly positive (++) phenols were noticed in ethanolic and 50% ethanolic extracts of *T.cordifolia* and *M.charantia* alongwith 50% ethanolic extract of *R.Sativus*. Ethanolic and 50% ethanolic extracts of *Cucurbita maxima* showed positive (+) reaction for phenolic compounds whereas *Raphanus sativus* (Absolute ethanolic) showed negative (-) reaction for phenols. Highly positive (++) reaction for saponins was recorded in ethanolic and 50% ethanolic extract of *T.cordifolia*.

Positive (+) reaction was noticed only in 50% ethanolic extract of *C.maxima* whereas ethanolic extract of *C.maxima* and both the ethanolic and 50% ethanolic extracts of *M. charantia* and *R.sativus* revealed negative (-) saponin reaction by foam test. Both extracts of *R. sativus* (ethanolic and 50% ethanolic) revealed highly positive (++) reaction for tannins. All other extracts showed positive (+) reaction for tannins.

TABLE 1: IN VITRO QUALITATIVE PHYTOCHEMICAL ANALYSIS OF VARIOUS ETHANOLIC (E) AND 50 % ETHANOLIC (A: E) EXTRACTS

Extract (s) /Test(s)	Alkaloids	Phenols	Saponins	Tannins
	Dragendroff's Test	FeCl ₃ Test	Foam Test	Gelatin Test
<i>T. cordifolia</i> (E)	-	++	++	+
<i>T. cordifolia</i> (A:E)	++	++	++	+
<i>M. chirantia</i> (E)	+	++	-	+
<i>M. chirantia</i> (A:E)	+	++	-	+
<i>C. maxima</i> (E)	-	+	-	+
<i>C. maxima</i> (A:E)	++	+	+	+
<i>R. Sativus</i> (E)	++	-	-	++
<i>R. sativus</i> (A:E)	-	++	-	++

(+) positive, (++) highly positive and (-) Negative

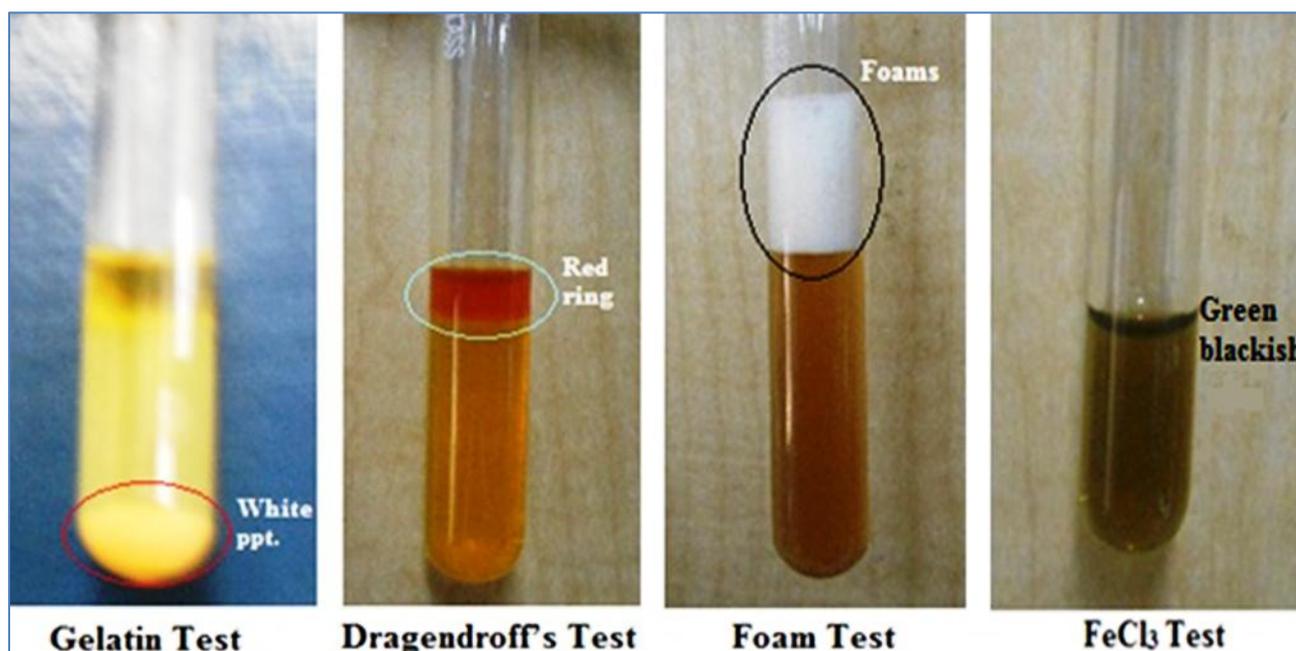


FIGURE 1: DIFFERENT QUALITATIVE PHYTOCHEMICAL TESTS FOR TANNINS, ALKALOIDS, SAPONINS & PHENOLS

Total flavonoid contents of the extracts were determined according to colorimetric method and expressed in mg of catechin equivalent per gm (mg CE/gm) of extract, by reference to standard curve ($y = 0.008x + 0.016$ and $R^2 = 0.927$). The presences of catechin content of extracts revealed

highest content in 50% ethanolic extract of *Tinospora cordifolia* (9.91 ± 1.46 mg CE/gm) followed by 50% ethanolic extract of *Cucurbita maxima* (9.79 ± 0.35 mg CE/gm) and ethanolic extract of *Tinospora cordifolia* (9.75 ± 0.4 mg CE/gm) among all the 8 tested extracts (Table 2).

Total phenol content, as determined by the Folin-Ciocalteu method, is reported as gallic acid equivalents (mg GAE/g) by reference to standard curve ($y = 0.045x - 0.006$ and $R^2 = 0.983$; where y is absorbance, X is concentration and R^2 is the coefficient of determination) as shown in Fig.3. Total phenolic content was significantly ($p < 0.05$)

higher in 50% ethanolic extracts of *Tinospora cordifolia* (24.70 ± 2.04 mg GAE/gm) followed by 50% ethanolic extract of *Cucurbita maxima* (18.23 ± 0.03 GAE/gm) and absolute ethanolic extract of *Tinospora cordifolia* (12.33 ± 0.5 mg GAE/gm) (Table 2).

TABLE 2: IN VITRO QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF DIFFERENT VEGETABLE/PLANT EXTRACTS (MEAN \pm SE)

Plant Extracts/ Test (s)	Total Phenols (mg GAE/gm)	Total Flavonoids (mg CE/gm)
<i>M. chirantia</i> (A:E)	11.54 ± 0.007^c	4.68 ± 0.10^{ab}
<i>C. maxima</i> (E)	7.34 ± 0.04^b	7.49 ± 0.20^{bc}
<i>C. maxima</i> (A:E)	18.23 ± 0.03^d	9.79 ± 0.35^c
<i>R. sativus</i> (E)	4.22 ± 0.06^{ab}	2.49 ± 0.32^a
<i>R. sativus</i> (A:E)	3.28 ± 0.29^a	1.82 ± 0.05^{ac}
<i>M. chirantia</i> (E)	4.53 ± 0.36^{ab}	7.26 ± 0.4^b
<i>T. cordifolia</i> (E)	12.33 ± 0.5^c	9.75 ± 0.4^c
<i>T. cordifolia</i> (A:E)	24.70 ± 2.04^e	9.91 ± 1.46^c

Values bearing different superscripts in the same column differ significantly ($P \leq 0.05$)

DISCUSSION: Previous study revealed the presence of alkaloids, phenols and saponins in absolute ethanolic, methanolic and acetone extracts of *Tinospora cordifolia* stems. However same author recorded negative (-) Mayer's test for hydroethanolic and aqueous extract of *Tinospora cordifolia* (stems) which is concurrent with present study⁹. Positive (+) reactions for Dragendroff's and Lead acetate test were recorded in hydroethanolic and aqueous extract of *Tinospora cordifolia* (stems) in earlier study¹⁰. In contrary to our reports, one study reported absence of saponin glycosides in *T. cordifolia* extracts¹⁰.

Fruit extract of *Momordica charantia* revealed presence of alkaloids, steroids, phenolic compounds, flavonoids tannins, anthraquinones and amino acids¹¹. One of previous *in vitro* phytochemical analysis revealed the presence of tannins, saponins, flavonoids, anthraquinones, carbohydrates, steroids, phytosterol, alkaloids, amino acids, terpenoids, cardiac glycosides and chalcones in *R. sativus*¹². In present study, phenolic content was negative in 50% ethanolic extract of *Raphanus sativus* which is in concurrence with earlier report who also revealed absence of phenolic compounds in *Raphanus sativus*¹². Negative (-) saponin reaction in aqueous fruit extracts of *R. sativus* and *M. charantia* revealed in earlier study supports present findings¹³.

One study reported the presence of chemical constituents like alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, steroids and phenolic compounds in Pumpkin (*Cucurbita maxima*) fruits¹⁴. Several reports confirmed the presence of phenolic compounds in fruits of *M. charantia* and *C. maxima*^{11, 14}.

Positive (+) ferric chloride test for *C. maxima* indicated presence of phenolic compounds in earlier study is in accordance with the present findings¹⁵. Earlier studies recorded negative (-) foam reaction in *C. maxima* and *M. charantia* extracts^{11, 15}. *R. sativus*^{12, 13}, *M. charantia*^{11, 12}, *T. cordifolia*^{9, 10} and *C. maxima*¹⁴ are reported for presence of tannins by several authors from time by time are in accordance with present findings.

Total phenol and Flavonoids contents (TPC/TFC) were high in 50% ethanolic extracts of *Tinospora cordifolia* and *Cucurbita maxima*. Earlier study revealed 12.8mg/gm of total phenolic contents in *T. cordifolia* extract however total flavonoid content recorded was 6mg/gm of *T. cordifolia* extract⁹. Total phenol content (TPC) of *T. cordifolia* grown on *Azadirachta indica* was found to be 21.5 mg/gm of dry weight of extract¹⁶. Our findings are in agreement with above authors. It is known that total phenol content is responsible for the free radical scavenging activity in many plants¹⁷.

Total polyphenolic, flavonoid and flavonol content of *Cucurbita maxima* found to be 12mg/gm (gallic acid equivalent), 3.8 mg/gm and 0.8 mg/gm (rutin equivalent) respectively. Quantification of phenolics in *Cucurbita maxima* by Folin-Ciocalteu method showed the presence of good amount of total phenolics (12mg/gm calculated as per gallic acid), total flavonoid and flavonol content found to be 3.8 mg/gm and 0.8 mg/gm (rutin equivalent) respectively¹⁸.

CONCLUSION: *In vitro* qualitative and quantitative phytochemical analysis of different vegetable/herb extracts revealed highest activity in 50% ethanolic extract of *T. cordifolia* > *C. maxima* (A:E) > *T. cordifolia* (E) among 8 different extracts.

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