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Exploration of bioactive compounds in *Operculina Turpethum* L.

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Abstract
A plant in the morning glory family, *Operculina turpethum (syn. Ipomoea turpethum)* is known commonly as *turpeth. Operculina turpethum* L. is a highly perennial medicinal plant of the family *Convolvulaceae*. Plant kingdom harbours an inexhaustible source of bioactive ingredients valuable in the management of many intractable diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Furthermore, the active components of herbal remedies have the advantages of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components. The use of medicinal plants to treat human disease has its roots in pre-historic times. A wide range of medicinal plants part is used for extract as raw drugs and they possess varied medicinal properties. Quantitative phytochemical analysis such as Alkaloids, Bitters, Saponins, Mucilage, and Tannins were conducted to evaluation of crude extracts of different plant parts such as leaf, stem, root, flower and fruit of *Operculina turpethum* L. Finding of the study of crude extracts of the *Operculina turpethum* L. revealed the quantification of Total Alkaloids, Bitters, Saponins, Mucilage, and Tannins. It equally ascertains the bioactive compounds in the plant, thus concluding with the potential therapeutic significance of the plant as a natural source.

Keywords: *Operculina Turpethum* L., phytochemical, bioactive compounds, secondary metabolites

Introduction
Trivrt consists of dried root of *Operculina turpethum* (Linn.) Silva Manso Syn. *Ipomoea turpethum* R. Br. (Fam. *Convolvulaceae*); a large perennial twiner with milky juice and fleshy roots, found growing wild nearly throughout the country, ascending to 900 m, also occasionally grown in gardens; the roots being fleshy, care is taken in drying as they decay easily. *Operculina turpethum* or Indian Jalap is widely grown throughout India and it is occasionally cultivated in gardens as an ornament.

The root bark of *Trivrit* is rich in *turpethin resin* consisting of 10% *'turpethin'* which is a glycoside analogue of *Jalapine* and *Convolvulin* and also contains *Turpethinic acids*- A, B, C, D, & E, *volatile oil, albumin, starch, lignin salts, ferric oxide, Scopoleptin, Betulin, lupiol & betasitosterol*. The roots are bitter, acrid, sweet, thermogenic, analgesic, purgative, carminative, antihelmintic, expectorant, antipyretic, hepatic, stimulant and hydragogue. (Sharma V and Singh M., 2012) [16, 18]. *Operculina turpethum* L. is among the most important purgatives in the *Indian materia medica*. It has been known in ayurveda as "*Virechan*" or a laxative.

The time- tested ethnic knowledge when supplemented with the latest scientific insights can offer new models of economic development, that are both eco-friendly and socially acceptable. (Croom E. M., 1983) [19] Recent research on phytochemicals is fervently focusing on health promotion, disease prevention, and the development of therapeutic interventions. The introduction of terms such as "functional food" and "nutraceutical" illustrates the high expectations associated with current phytochemical research. (Mark *et al*., 2004) [10]

Plants are the main source of substance and medications to the human kind since ages. The medical tradition of India is perhaps the most ancient spanning form the prehistoric times. Just as there is continuity of life, there is continuity of medical science. It is natural to look upon the Vedic literature as a channel through which this continuous medical tradition reached down to the earliest systemetisers. Vedic Samhitas contain abundant references, relating to both diseases and drugs of plant origin.

Hence, the qualitative screening of the plant for the presence of various phytochemicals finds its relevance to provide the source of potent natural remedy.

Material & Methods

For the correct interpretation, it is essential that plant material's sampling is done at the prescribed morphological stage of growth and the correct plant parts. Fresh plant tissues should be used for analysis and the material should be free from contaminations. Different plant parts of *Operculina Turpethum* L. were collected from nursery stock maintained at in house garden at own residential plot. Plant parts were manually cleaned and shed dried under natural condition for further analysis.

Different plant parts of *Operculina Turpethum* L. such as leaf, root, stem, fruit and flower's dried powdered materials were used in the soxhlet thimble to obtain sequential extracts of different solvents aqueous, hydro-alcoholic (methanol 50% v/v) and alcoholic (methanol). The materials were refluxed with each solvent for 4-6 hours for 4 extractions cycle at 60-80 ºC. Pool extraction washing, filtered and distill off solvents under reduced pressure. Now, soft extracts were collected and cooled at room temperature and poured in glass Petri dishes & then evaporated at 40-60 ºC using vacuum oven. Dried extracts were used for further study and remaining was stored at 5 ºC in air tight containers.

The calculation of the extraction yield was the weight percentage of the crude extract to the raw material (500 gm). The percent extraction yield was calculated as follows.

% Extraction yield = Weight of the plant extract/Weight of the initial sample x 100%

Different plant parts of *Operculina Turpethum* L. were evaluated through the well established procedures of phytochemical analysis like Total Alkaloids (*Indian Pharmacopoeia*, 2010) [10], Bitters, Saponins, Tannins and Mucilage content. (*Standardization of Botanicals*, Vol. 1 and 2)

Total Alkaloids

Shake about 20 g of the extract under examination with 400 ml of a solution containing 4 volumes of *ether* and 1 volume of *ethanol*. Add 20 ml of 5 per cent v/v *ammonia solution* into 1000-ml conical flask and shake for one hour. Decant and filter through cotton. Transfer the filtrate into a separator. Wash the residue with a mixture containing 80 volumes of *ether* and 20 volumes of *ethanol*. To the filtrate add 100 ml of *0.5 M sulphuric acid.* Collect the lower layer into another separator. To the ether layer; add 100 ml of a mixture containing 80 volumes of *0.25M sulphuric acid* and 20 volumes of *ethanol* and extract. Continue the extraction 3 times with 80 ml of the *0.25 M sulphuric acid* and a mixture containing 80 volumes of *ether* and 20 volumes of *ethanol* until aqueous layer is colourless. Combine the acid solution and wash with 40 ml of *chloroform* followed by 2 times with 20 ml of *chloroform.* Wash, the combined chloroform layer, with acid alcohol mixture. Discard the chloroform layer. Combine the acid alcohol solutions and make it alkaline with 5 per cent v/v *ammonia solution* and add 10 ml excess. Extract 3 times with 100 ml of *chloroform.* Add 2 ml of *0.1 M hydrochloric acid* to 0.5 ml of the extract, remove the *chloroform* by evaporation, transfer the aqueous residue to a test tube and add 0.05 ml of *potassium mercury-iodide solution;* not more than a very faint opalescence is produced. If the opalescence is more, the extraction is not complete. Extract further 2 times with 100 ml of *chloroform.* Combine the chloroform extract and wash with 20 ml of *distilled water*. Filter the chloroform layer through cotton plug into a tared beaker. Wash the residue with a little chloroform, transferring the chloroform layer to the same tared beaker. Evaporate on a waterbath. Add 5 ml of *alcohol* to the residue and dry to constant weight at 105 °C. Finally weigh the residue and calculate the content of total alkaloids.

Bitters

Reflux 3 gm extract with 50 ml of *alcohol* on a water bath for water bath for half an hour and filter. Repeat the above process twice or till bitterness is observed in residue. Evaporate the alcohol under vacuum from the filtrate and shake the residue repeatedly with 25, 15, and 15 ml of *hot water*. Shake the above aqueous extract repeatedly with 25, 20, 15 and 15 ml of *Ethyl Acetate*, and evaporate to dryness and weigh.

Saponins

Take 5gm Sample extract with 90% v/v *methanol* (25 ml) by refluxing for half an hour. Extract the residue two more times by taking 25 ml methanol. Combine methanolic extract and distil off the solvent. Treat the soft extract left after distillation of alcohol, with 25ml *petroleum ether 60-80 ˚C*, 25 ml by refluxing for half an hour. Cool and remove the solvent by decantation. Now treat the same soft extract successively with *chloroform* 25 ml and *ethyl acetate* 25 ml and pour the solvents after cooling, keeping the soft extract in the same flask. Dissolve the soft extract (after three extractions cited above) in 25 ml of 90% v/v *methanol*. Filter and concentrate to 5 ml. Add above drop by drop with constant stirring to 25 ml acetone in order to precipitate saponins. The precipitates are filtered, collected and dried to constant weight at 105 °C.

Tannins

Take 1 g of extract in 100 ml of vol. flask & add 50 ml of *Hot water* & make up volume up 100 ml & then filter by 41 no. filter paper &, transfer 10 ml of filtrate to a conical flask 1Ltr. capacity, and add 750 ml of water and 25 ml of *indigosulphonic acid* solution and titrate with constant stirring against *N/10 Potassium permaganate* solution to a golden yellow colour.

1 ml of *N/10 Potassium permanganate* is equivalent to 0.004157 g of tannin compounds calculated as tannic acid. Run a blank test by titrating 25 ml of *indigosulphonic acid* in 750 ml of water.

Preparation of *indigosulphonic acid solution*, dissolve 1 g of *Indigo carmine* in 25 ml of concentrated *sulphuric acid*, add another 25 ml of *concentrated sulphuric acid* and dilute with distilled water to1 Ltr. cautiously pouring the solution into the *water*.

Mucilage

Take 5 g sample of extract powder in a conical flask. Add 100 ml *water* and shake well for two hours. Leave overnight. Next day, filter and concentrate up to 10 ml. Add 50 ml 90% v/v *methanol* with constant stirring and leave it for half an hour. Filter and dry the residue on filter paper at 80 °C under vacuum till constant weight.

Results and Discussion

The yield of plant is mainly dependent on the type of solvent used in the extraction procedure. Table 1 and Graph 1 represents the % yield of different plant parts of *Operculina Turpethum* L. in different solvents. The values were high in

hydro-alcoholic extract of fruit, flower, root and stem where leaf was got higher yield through alcoholic extraction.

Operculina Turpethum L. is well widely used medicinal plant since ancient time and all most all the plant parts are used as natural remedies. Result of phytochemical analysis was summarized in Table 2, Graph 2-6. Total Alkaloids and Bitters content were derived higher in alcoholic solvent than other extraction solvent in all plant parts. Saponins content was notified higher in hydroalcoholic solvent than other extraction solvent in all plant parts. And Mucilage and Tannins content were measured higher in aqueous solvent than other extraction solvent in all plant parts of *Operculina Turpethum* L.

Table 1: Extraction yield of different extracts of different plant parts of *Operculina Turpethum* L.

Plant Parts	Type Of Extraction	% OF YIELD
Fruit	Aqueous extract	10.92%
	Hydro-alcoholic extract	11.85%
	Alcoholic extract	9.10%
Flower	Aqueous extract	8.59%
	Hydro-alcoholic extract	10.80%
	Alcoholic extract	9.65%
Leaf	Aqueous extract	8.67%
	Hydro-alcoholic extract	13.76%
	Alcoholic extract	14.62%
Root	Aqueous extract	10.25%
	Hydro-alcoholic extract	13.40%
	Alcoholic extract	11.50%
Stem	Aqueous extract	9.86%
	Hydro-alcoholic extract	13.10%
	Alcoholic extract	12.30%

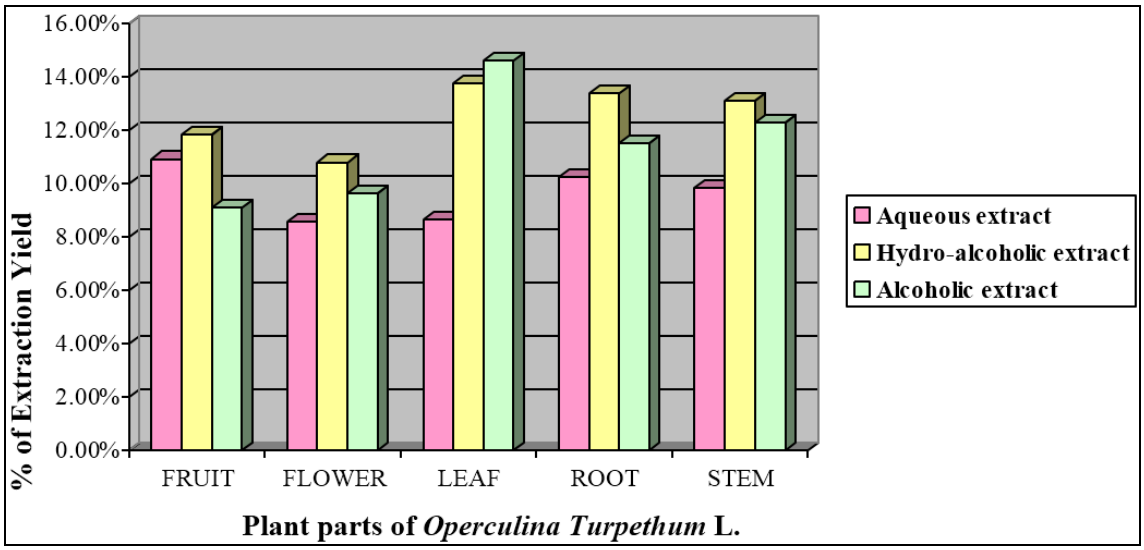

Fig 1: Extraction yield of different extracts of different plant parts of *Operculina Turpethum* L.

Table 2: Analysis of Bioactive constitutes of different plant parts of *Operculina Turpethum* L.

Plant Parts	Type of Extraction	Alkaloids	Bitters	Saponins	Mucilage	Tannins
		Active Constitutes				
FRUIT	Aqueous extract	0.11%	0.24%	7.65%	9.50%	8.36%
	Hydro-alcoholic extract	0.19%	1.05%	8.85%	7.45%	6.25%
	Alcoholic extract	0.23%	1.30%	6.50%	5.13%	4.25%
FLOWER	Aqueous extract Hydro-alcoholic extract	0.10% 0.15%	0.18% 0.72%	6.20% 8.14%	8.45% 6.91%	7.54% 5.65%
	Alcoholic extract	0.19%	0.97%	5.63%	5.46%	3.26%
LEAF	Aqueous extract	0.16%	0.24%	7.65%	8.60%	8.36%
	Hydro-alcoholic extract	0.28%	1.05%	8.85%	7.45%	6.25%
	Alcoholic extract	0.38%	1.30%	6.50%	5.13%	4.25%
ROOT	Aqueous extract	0.18%	0.36%	8.43%	12.50%	11.72%
	Hydro-alcoholic extract	0.26%	1.95%	9.17%	9.48%	8.95%
	Alcoholic extract	0.45%	2.63%	7.75%	7.85%	7.45%
STEM	Aqueous extract	0.13%	0.31%	7.85%	10.7%	9.40%
	Hydro-alcoholic extract	0.25%	1.40%	8.62%	8.96%	7.05%
	Alcoholic extract	0.38%	2.10%	7.15%	7.22%	6.47%

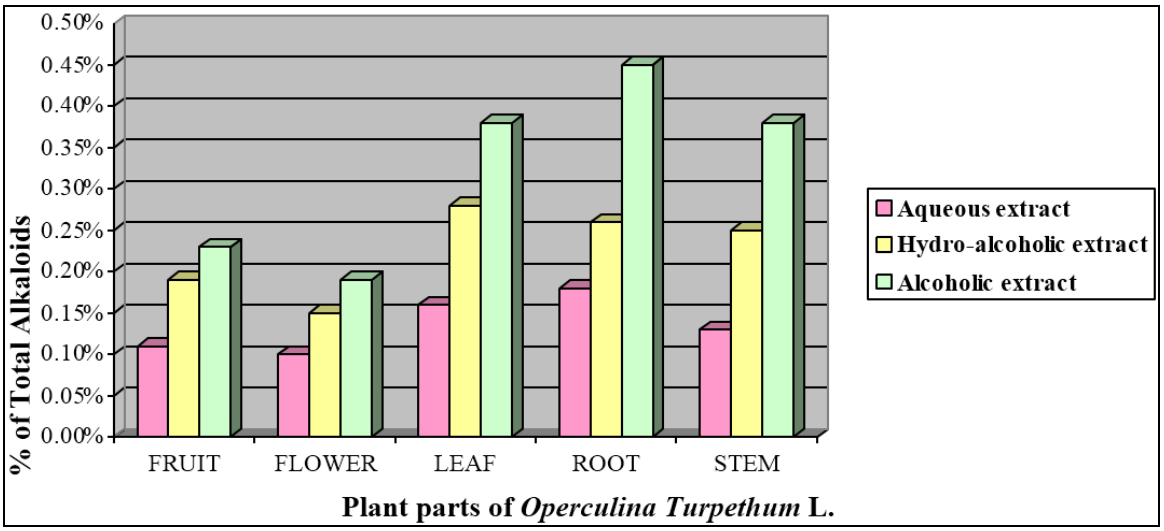

Fig 2: Determination of Total Alkaloids content of different extracts of different plant parts of *Operculina Turpethum* L.

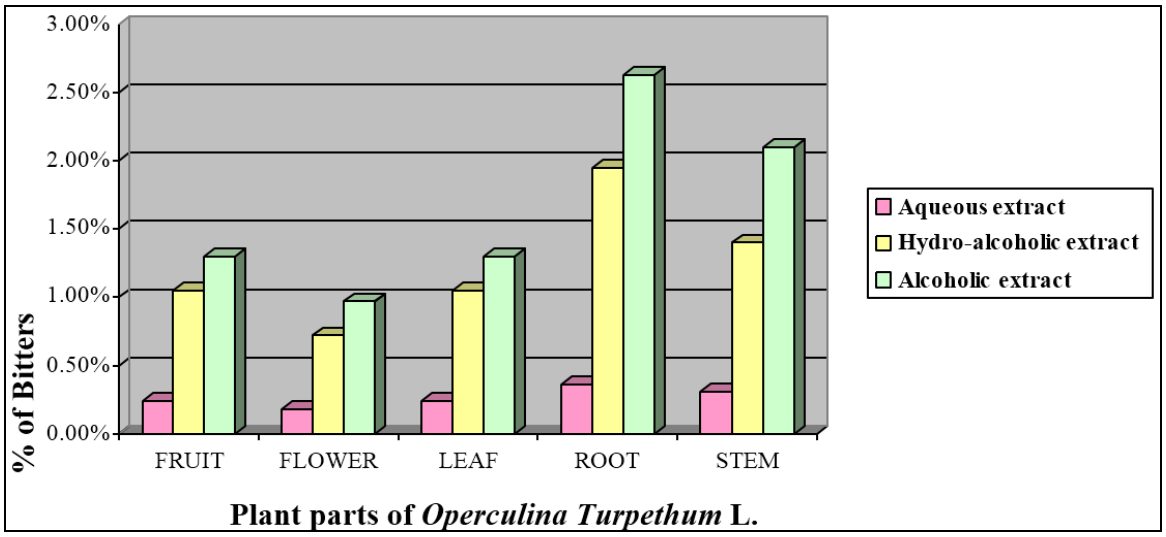

Fig 3: Determination of Bitters content of different extracts of different plant parts of *Operculina Turpethum* L.

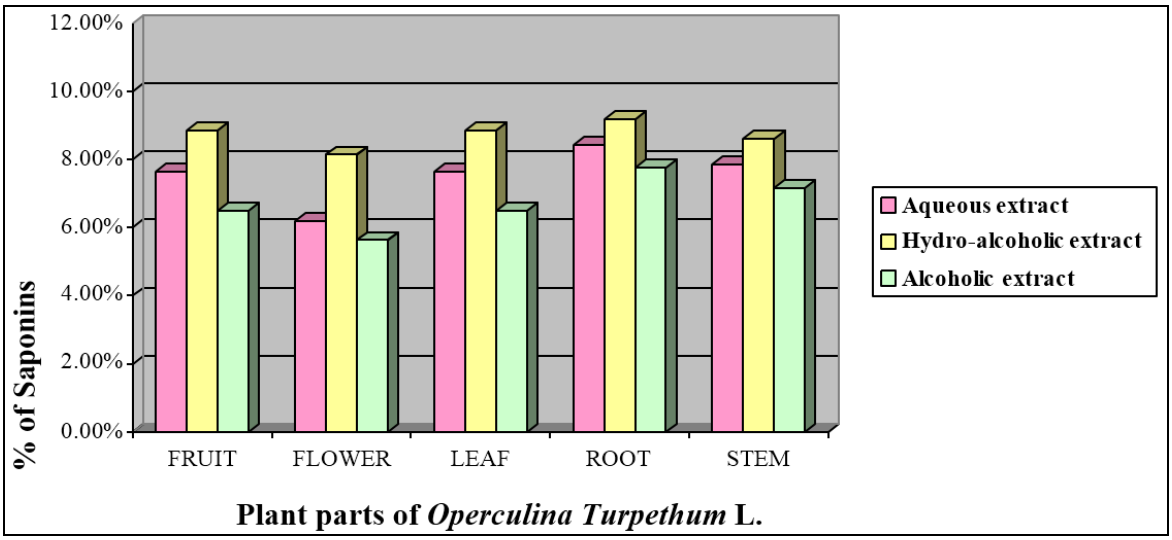

Fig 4: Determination of Saponins content of different extracts of different plant parts of *Operculina Turpethum* L.

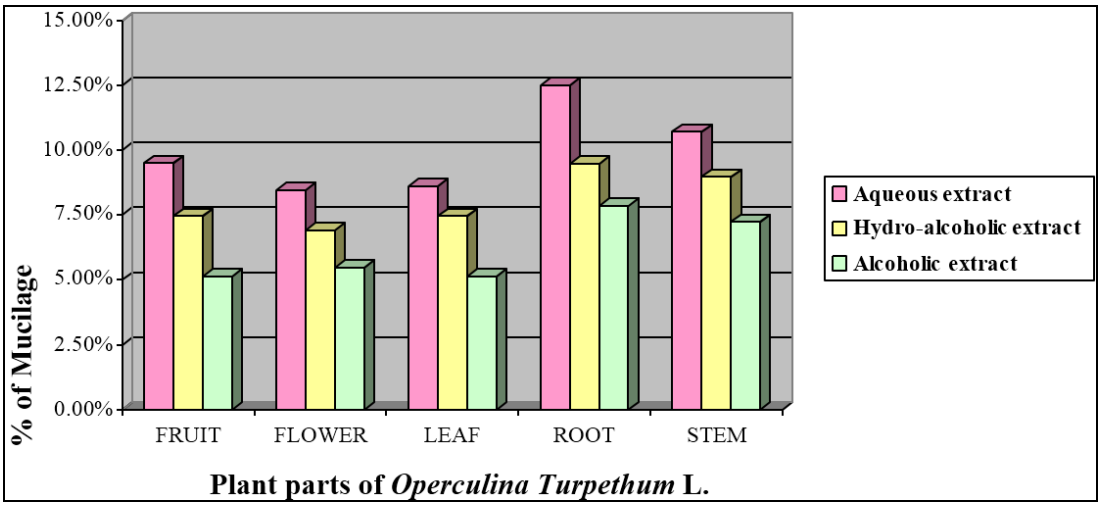

Fig 5: Determination of Mucilage content of different extracts of different plant parts of *Operculina Turpethum* L.

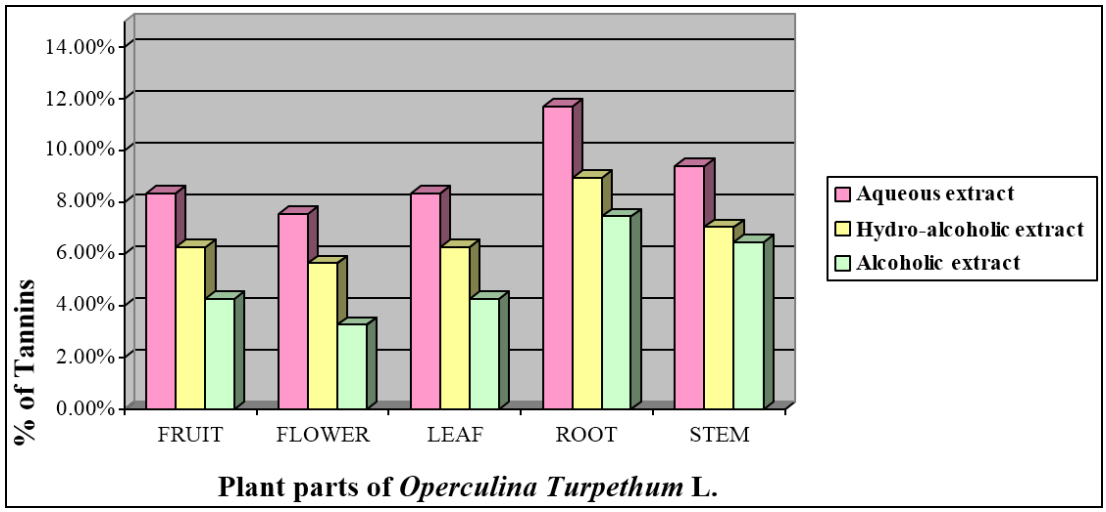

Fig 6: Determination of Tannins content of different extracts of different plant parts of *Operculina Turpethum* L.

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents is generally provided by the phytochemical screening of plant extracts. In the present study, quantitative tests for all plant parts showed significant indication about the presence of bioactive compounds. This finding of phytochemicals is good enough to reflect their importance.

The quantified phytochemical analysis revealed the presence and quantification of Alkaloids,

Bitters, Saponins, Mucilage and Tannins in different extracts of different plant parts of *Operculina turpethum* L. The study apparently highlighted the scientific basis for the possible use of *Operculina turpethum* L. in ethno-medication.

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