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The Pharmacognostic and Proximate Composition Analysis from Leaf part of Genus *Grewia*

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Abstract

The quantitative pharmacognostic properties and nutritional composition from species of genus Grewia were studied using leaf powder sample. Several *Grewia* species are well familiar for their ethnobotanical importance and therapeutic properties. Any plant which possesses medicinal properties should be subjected to detailed pharmacognostic study so that its proper identification can be done. The perspective of present study was to carry out the pharmacognostic determination viz., Sugars, Methanol, Ethanol, Pet ether, Acetone, Chloroform and Benzene as well as the biochemical analysis viz., dry matter, crude protein, crude fat, crude fibre, cellulose, carbohydrates, total ash and gross energy of the plant parts using leaf powder samples of ten species of *Grewia* (*G. asiatica, G. abutilifolia, G. damine, G. flavescence, G. aurantifolia, G. nervosa, G. serrulata, G. orbiculata, G. villosa, G. tenax and G. tilifolia)* from Western Maharashtra region.

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Keywords: Pharmacological properties, Ethanol, Methanol, pet ether, Acetone, Chloroform, Proximate composition, Nutrients, bioactive compounds.

INTRODUCTION:

The fruit, leave, bark of *Grewia* species have high medicinal values and are widely used for the treatment of various common diseases and presence of different metabolites like saponins, coumarins and anthraquinone (Sharma and Patni, 2013). The leaves of *Grewia asiatica* have been reported to possess various pharmacological activities such as anti-malarial, anti-emetic, anti-platelet, antimicrobial, anti-diabetic and anti-cancer activities (Zia-Ul-Haq *et al.,* 2012; Sangita *et al*., 2009; Parveen *et al.,* 2012 and Kakoti *et al.,* 2011). In *G. tiliaefolia*, the ethanolic extract of aerial parts exhibited CNS depressant and diuretic activity while that of stem bark exhibited spermic and hypotensive activity (Dhawan *et al.,* 1977). The *Grewia villosa* is also known to contain harman alkaloids. Harman alkaloids belong to the class of β-carbolines and bind strongly to receptors in the brain and affect the CNS (Pfau and Skog, 2004). *G. tenax* is a plant that has been used in popular medicines in various ways in different countries for cure of jaundice, pulmonary infections, and asthma. Methanolic crude extract of G. tiliaefolia was checked against different radical systems comprising superoxide radical (O22), hydroxyl radical (OH), and nitric oxide radical (NO) and showed potential anti-oxidant activity in the in vitro model system (Selvan *et al.,* 2010). *Grewia tiliaefolia* contain chemicals like D-erythro-2-hexenoic acid γ-lactone, Gulonic acid γlactone, Betulin, Friedelin, Lupeol, Tannins, Flavonoids, Hemicelluloses, Phenolics, Lupeol, and Lignin (Kumar and Venkatachalam, 2017; Badami *et al*., 2004; Anjaneyulu *et al.,* 1965; Badami *et al.,* 2003 and Goyal, 2012). *Grewia tenax* and *Grewia villosa* were identified as food producing species and that they are consumed during time of food shortage (Tahir *et al.,* 2004), despite this they are underutilized (Looy *et al.,* 2008). The extracts from the plant of *Grewia* are known to have medicinal properties (Rosa *et al.,* 2006). The presence of various biofunctional and chemo-preventive compounds in different parts of plant, believed to have health-boosting properties, are a major reason for their increased consumption and the medicinal plants have always been an exemplary source (Kaur and Kapoor, 2005).

MATERIAL AND METHODS:

To carry out the pharmacognostic and proximate determination the species of genus *Grewia viz., Grewia asiatica, Grewia abutilifolia, Grewia damine, Grewia flavescence, Grewia aurantifolia, Grewia nervosa, Grewia serrulata, Grewia orbiculata, Grewia villosa, Grewia tenax and Grewia tilifolia* were selected. The plant samples were collected from different localities of Maharashtra state viz., Sangli, Satara, Sholapur, Kolhapur and Pune. The material was collected in polythene bags and brought to the laboratory for pharmacognostic and proximate composition study. The powdered leaves were extracted with different solvents viz., Water, Methanol, Ethanol, Petroleum ether, Acetone, Benzene and Chloroform.

Determination of water soluble Extractive

The powder (5gm) of air dried sample was macerated with 100ml Distilled water in a closed flask, for 24 hrs, shaking frequently solutions was filtered and filtrated was evaporated in pre weighed taredflate bottom shallow dish, further dried at 100oC and weighed. The % of water soluble extractive was calculated.

Determination of Acetone soluble Extractive

The powder (5gm) of air dried sample was macerated with 100ml Acetone in a closed flask, for 24 hrs, shaking frequently solutions was filtered and filtrated was evaporated in pre weighed taredflate bottom shallow dish, further dried at 100oC and weighed. The % of water soluble extractive was calculated.

Determination of Benzene soluble Extractive

The powder (5gm) of air dried sample was macerated with 100ml Distilled water in a closed flask, for 24 hrs, shaking frequently solutions was filtered and filtrated was evaporated in pre weighed taredflate bottom shallow dish, further dried at 100oC and weighed. The % of water soluble extractive was calculated.

Determination of Ethanol soluble Extractive

The powder (5gm) of air dried sample was macerated with 100ml Ethyl alcohol in a closed flask, for 24 hrs, shaking frequently solutions was filtered and filtrated was evaporated in pre weighed taredflate bottom shallow dish, further dried at 100oC and weighed. The % of Ethyl Alcohol soluble extractive was calculated.

Determination of Methanol soluble Extractive

The powder (5gm) of air dried sample was macerated with 100ml Methanol in a closed flask, for 24 hrs, shaking frequently solutions was filtered and filtrated was evaporated in pre weighed taredflate bottom shallow dish, further dried at 100oC and weighed. The % of Methanol soluble extractive was calculated.

Determination of Petroleum ether soluble Extractive

The powder (5gm) of air dried sample was macerated with 100ml Pet Ether in a closed flask, for 24 hrs, shaking frequently solutions was filtered and filtrated was evaporated in pre weighed taredflate bottom

shallow dish, further dried at 100oC and weighed. The % of Petroleum ether soluble extractive was calculated.

Determination of Dry Matter: The dry matter was determined on the basis of the AOAC method. The dry matter was calculated by following equation.

$$\textbf{Dry matter \% =} \quad \frac{\text{weight of dry matter}}{\text{Fresh weight of sample}} \times 100$$

Determination of Total protein (Nitrogen): The protein content was determined from the organic Nitrogen content by Kjeldahl method. The protein content was calculated by following equation.

Protein content = 1 ml of 0.1 N H_2SO_4 = 0.0014gm N. Calculate protein as N × 6.25 Protein on dry wt. basis = Protein content x 100 (100–Moisture content)

Determination of Crude fiber: The determination of crude fibre was based on the basis of AOAC (1970) method.

$$\textbf{Crude fibre (\%) =} \quad \frac{\text{(Dry weight of digested sample - Weight of ash)}}{\text{Weight of sample}} \times 100$$

W1 = Wt in gm of gooch crucible and contents before ashing

W2 = Wt in gm of gooch crucible containing asbestos and ash

W = Wt in gm of the dried material taken for the test

Calculated crude fiber on dry wt. basis by giving correction for the moisture content

Determination of Crude Fat: The ash content was determined by AOAC (1970) method. The sample of oil seed was grinded and taken on filter paper, weighed it, the weight was noted. The thimble was made. The thimble was wrapped with thread and put the thimble in extraction set. 200 ml solvent (Hexane) was added and kept the extraction set in heating mental and heated for 8-10 hrs. The solvent was recovered after 8-10 hrs from flask and kept the oil bearing flask in oven at 130Oc, after completed drying, cooled the flask and weighed up to constant weight.

$$\textbf{Crude} \text{ Fat (\%) =} \quad \frac{\text{Weight of the sample}}{\text{Weight of fat}} \times 100$$

Determination of Total Ash: The ash content was determined by AOAC (1970) method. The dried material was ignited in the dish left after the determination of moisture with the flame of a burner till charred. It was transferred to a muffle furnace maintained at 550 - 600ºC and ignition was continued till grey ash was obtained. It was cooled in a desiccator and weight was measured. The process was repeated for heating, cooling and weighing at half hour interval till the difference in weight in two consecutive weighing was less than 1 mg. The lowest weight was recorded.

$$\textbf{Total Ash (\%) =} \quad \frac{\text{(W2-W1)}}{} \times 100$$

W1-W

Where
W2 = Weight in gm of the dish with the ash
W = Weight in gm of empty dish
W1 = Weight in gm of the dish with the dried material taken for test.

Determination of Cellulose: The cellulose content was determined by AOAC (1970) method. The cellulose content was calculated from standard graph and optical density taken of sample.

Determination of Carbohydrates: Total carbohydrate (TC) was determined by differential method of (Janardhanan and Lakshmanan, 1985). This was achieved by subtracting the total protein, lipid, moisture and ash content from 100.
% TC = % NFE + % CF or
% TC = 100- (% CP + % Cfat + % ash)

GROSS ENERGY (GE):
The chromic acid oxidation method described by O'shea and Maguire (1962) was followed to determine gross energy (GE) and the amount of GE was determined and calculated in Kcal per g of sample using the following equation:

$$\textbf{GE (Kcal/g DM)} = \frac{\text{ml 1.5 N } K_2Cr_2O_7 \text{ used to oxidize 1g sample}}{(23.39 - 0.069\ P + 0.000226\ P^2)}$$

Where P is the crude protein (CP) the content in the sample expressed as per cent of dry matter (DM).

RESULTS AND DISCUSSION:

The pharmacognostic study revealed that in *Grewia abutifolia* the values of sugar, non-reducing sugar and total sugar were 2.33%, 1.1% and 3.41% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 27.86%, 19.3%, 11.9%, 18.1%, 12.9%, 10.86% and 7.43% respectively. The results are shown in the Table No. 1. The values of sugar, non-reducing sugar and total sugar were 1.48%, 0.33% and 1.81% respectively. Whereas the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 23.00%, 19.01%, 11.48%, 23.03%, 13.00%, 11.09% and 6.99% respectively in *Grewia asiatica.* In *Grewia damine* the values of sugar, non-reducing sugar and total sugar were 5.3%, 0.90% and 6.02% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 21.00%, 18.6%, 13.2%, 25.5%, 12.5%, 10.6% and 8.04% respectively. The results of tests carried out for solvent extraction from leaf powder sample in *Grewia flavescence* showed that the values of sugar, non-reducing sugar and total sugar were 1.3%, 1.1% and 2.42% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 30.24%, 22.24%, 10.98%, 31.24%, 14.76%, 15.69% and 7.52% respectively. The study revealed that in *Grewia nervosa* the values of sugar, non-reducing sugar and total sugar were 0.79%, 0.36% and 1.15%

respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 22.9%, 18.93%, 12.2%, 9.9%, 13.78%, 11.86% and 6.34% respectively. The study performed on leaves to find out pharmacognostic attributes showed that from *Grewia orbiculata* the values of sugar, non-reducing sugar and total sugar were 2.1%, 1.1% and 3.2% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 20.44%, 19.61%, 12.6%, 16.6%, 12.6%, 11.4% and 7.48% respectively. In *Grewia serrulata* the values of sugar, non-reducing sugar and total sugar were 1.81%, 0.67% and 2.47% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 24.84%, 20.02%, 14.09%, 15.9%, 13.3%, 12.00% and 8.90% respectively. In *Grewia tenax* the values of sugar, non-reducing sugar and total sugar were 2.02%, 3.1% and 5.12% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 22.6%, 16.76%, 7.84%, 8.64%, 11.04%, 11.46% and 6.68% respectively. In *Grewia tilifolia* the values of sugar, non-reducing sugar and total sugar were 2.41%, 0.9% and 3.32% respectively. While the soluble extractive values of solvents viz., water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 24.68%, 21.96%, 13.68%, 26.04%, 12.9%, 14.76% and 8.8% respectively. Different solvents extracted from *Grewia villosa* indicated the values of water, methanol, ethanol, petroleum ether, chloroform, acetone and benzene were 23.62%, 18.44%, 9.36%, 10.3%, 13.48%, 10.92% and 8.28% respectively while the values of sugar, non-reducing sugar and total sugar were 3.03%, 0.87% and 3.9% respectively. The extractive values were also reported by (Maher kumar Gupta *et al.,* 2006) from fruits of *Grewia asiatica.* As depicted in the table No.2, the *G.abutifolia* contained DM (94.1%), ash (6.9%), nitrogen (2.03%), CP (12.69%), fat (2.82%), CF (32.30%), carbohydrates (39.4%), cellulose (28.8%) and GE(233kcal) on the basis of percent dry matter yield. The result of the biochemical analysis revealed that *G. asiatica* showed amount of DM, ash, nitrogen, CP, crude fat, CF, carbohydrate, cellulose and GE (kcal) in the percentage of 93.75%, 6.30%, 2.80%, 17.50%, 2.60%, 38.33%, 29.00%, 23.30% and 209 (kcal) respectively. Earlier work was carried out by (Toukhey *et al.* 2005) who recorded values for DM and CP from some two *Grewia* species. The concentration of DM, ash, nitrogen, CP, crude fat, CF, carbohydrate, cellulose and GE(kcal) from *G. damine* recorded was 93.50%, 5.43%, 2.24%, 14.00%, 2.55%, 29.10%, 42.40%, 26.6% and 249 (kcal) respectively. In response to proximate determination, the *Grewia flavescence* has the amount of DM (94.25%), ash (12.87%), nitrogen (2.00%), CP (12.51%), fat (3.65%), CF (36.30%), carbohydrates (28.90%), cellulose (30.23%) and GE (198kcal). The result of biochemical analysis based on the DM percentage of *Grewia nervosa* revealed that the values of DM, ash, nitrogen, CP, crude fat, CF, carbohydrate, cellulose and GE (kcal) were 93.30%, 8.00%, 2.07%, 12.96%, 3.86%, 29.10%, 38.60%, 31.20% and 240 (kcal) respectively. The results of Singh *et al*. (2004) showed that the values for Cp were greater in leaves of *Grewia* plants. From the leaf powder analysis based on the DM of *Grewia orbiculata* the percentage values were recorded of DM, ash, nitrogen, CP, crude fat, CF, carbohydrate, cellulose and GE (kcal) and that were 94.50%, 12.67%, 2.10%, 13.15%, 3.12%, 34.44%, 31.10%, 29.20% and 204 (kcal) respectively. The Table No. 2, shows that the *Grewia serrulata* has values for DM (95.00%), total ash (6.48%), nitrogen (2.71%), CP (16.86%), crude fat (2.75%), CF (24.44%), carbohydrate (44.40%), cellulose (29.90%) and the GE (270kcal). Papanastasis *et al.,* (2008) recorded higher ash content from *Grewia* fodder species. Similarly, results from *Grewia tenax* were DM (94.00%), ash (11.48%), nitrogen (3.02%), CP (18.92%), fat (3.64%), CF (31.40%), carbohydrates (28.60%), cellulose

(22.20%) and GE (222kcal). The results also indicated that *G. tilifolia* consisted of DM (94.50%), ash (7.93%), nitrogen (2.20%), CP (13.76%), fat (3.32%), CF (29.30%), carbohydrates (40.10%), cellulose (24.40%) and GE (245kcal). The table indicated that the proportion of DM, ash, nitrogen, CP, crude fat, CF, carbohydrate, cellulose and GE (kcal) in *G. villosa* was 94.50%, 12.67%, 2.10%, 13.15%, 3.12%, 34.44%, 31.10%, 29.20% and 204 (kcal) respectively. The present study revealed that *Grewia serrulata* yielded highest amount of DM (95.00%) amongst all other species. The highest concentration of total ash (12.87%) was found in *G. flavescence* compared to other species. *G. tenax* showed maximum concentration of nitrogen (3.02%) over all other species of *Grewia*. The CP (18.92%) was found to be highest in *Grewia tenax* as compared to other species. *Grewia nervosa* yielded maximum amount of crude fat (3.86%) in proximate study. The conc. of CF was recorded the maximum in *Grewia asiatica* (38.33%). During the proximate study the carbohydrate was found to be the maximum in *Grewia serrulata* (44.40%). The amount of cellulose (41.10%) was measured to be the highest in *Grewia villosa* while the GE was recorded to be the maximum in the species *Grewia serrulata* (270).

Table No. 1: Presence of sugars and soluble solvent extracts from leaf samples of *Grewia* species.

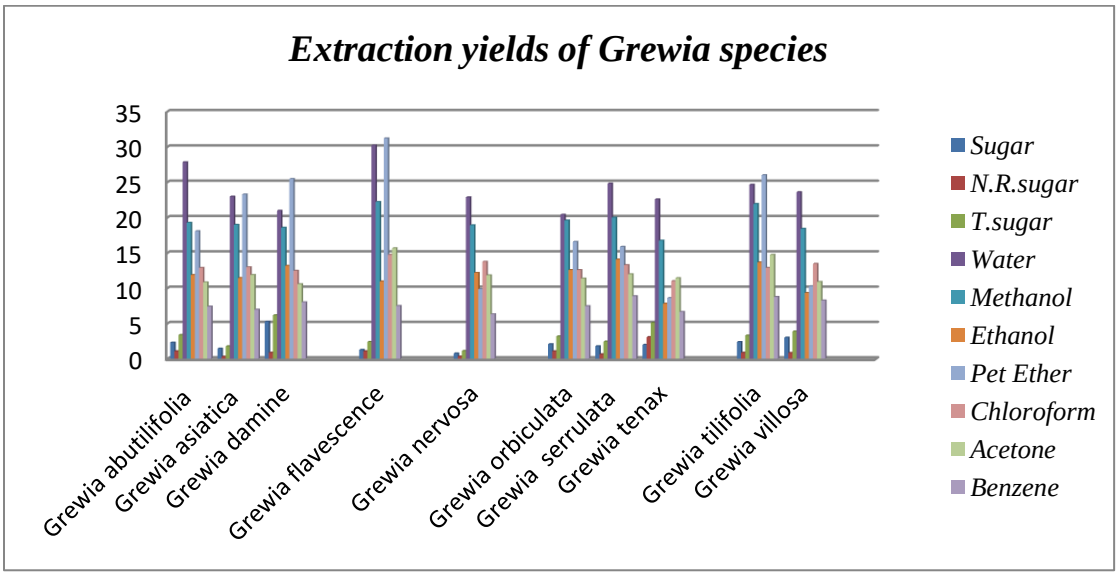
Plant Name	Sugar (%)	N.R.sugar(%)	T.sugar (%)	Water (%)	Methanol (%)	Ethanol (%)	Petroleum Ether (%)	Chloroform (%)	Acetone (%)	Benzene (%)
Grewia abutilifolia	2.33	1.1	3.41	27.86	19.3	11.9	18.1	12.9	10.86	7.43
Grewia asiatica	1.48	0.33	1.81	23.00	19.01	11.48	23.3	13.00	11.9	6.99
Grewia damine	5.3	0.90	6.2	21	18.6	13.2	25.5	12.5	10.6	8.04
Grewia flavescence	1.3	1.1	2.42	30.24	22.24	10.98	31.24	14.76	15.69	7.52
Grewia nervosa	0.79	0.36	1.15	22.9	18.93	12.2	9.9	13.78	11.86	6.34
Grewia orbiculata	2.1	1.1	3.2	20.44	19.61	12.6	16.6	12.6	11.4	7.48
Grewia serrulata	1.81	0.67	2.47	24.84	20.02	14.09	15.9	13.3	12	8.9
Grewia tenax	2.02	3.1	5.12	22.6	16.76	7.84	8.64	11.04	11.46	6.68
Grewia tilifolia	2.41	0.9	3.32	24.68	21.96	13.68	26.04	12.9	14.76	8.8
Grewia villosa	3.03	0.87	3.9	23.62	18.44	9.36	10.3	13.48	10.92	8.28

(R. Sugar = Reducing Sugar, **N. R. Sugar =** Non Reducing Sugar and **T. Sugar =** Total Sugar**).**

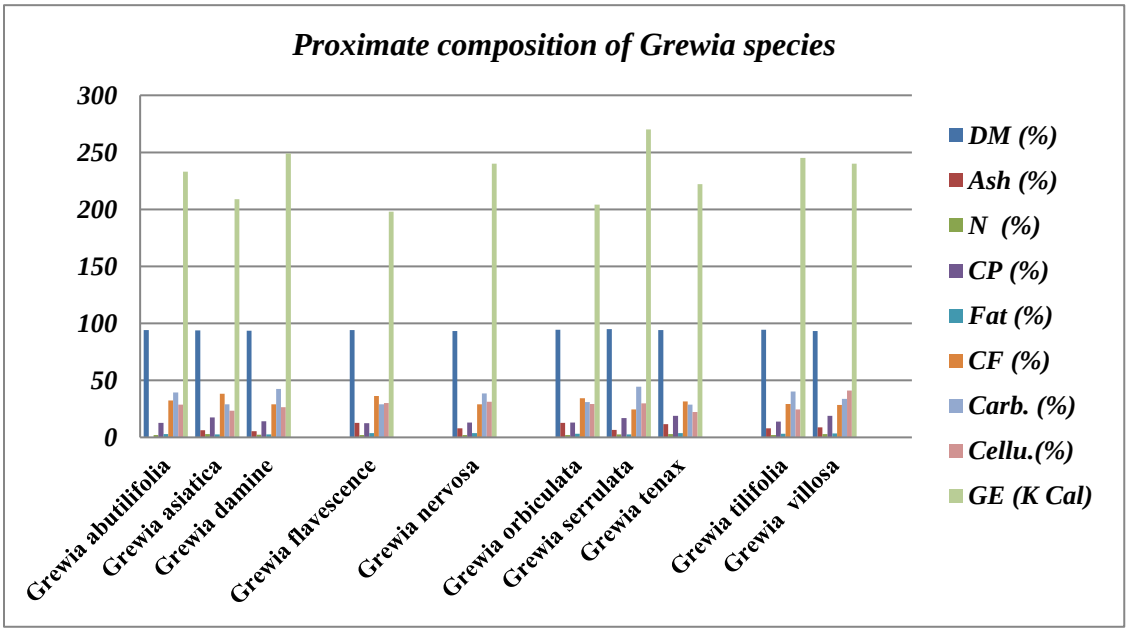
Table No. 2: Proximate composition of different species of genus *Grewia*

Plant Name	DM (%)	Ash (%)	N (%)	CP (%)	Fat (%)	CF (%)	Carb. (%)	Cellu .(%)	GE (K Cal)
Grewia abutilifolia	94.10	6.9 0	2.03	12.69	2.82	32.30	39.40	28.80	233
Grewia asiatica	93.75	6.30	2.80	17.50	2.60	38.33	29.00	23.30	209
Grewia damine	93.50	5.43	2.24	14.00	2.55	29.10	42.40	26.6	249
Grewia flavescence	94.25	12.87	2.00	12.51	3.65	36.30	28.90	30.23	198
Grewia nervosa	93.30	8.00	2.07	12.96	3.86	29.10	38.60	31.2	240
Grewia orbiculata	94.50	12.67	2.10	13.15	3.12	34.44	31.10	29.20	204
Grewia serrulata	95.00	6.48	2.71	16.86	2.75	24.44	44.40	29.90	270
Grewia tenax	94.00	11.48	3.02	18.92	3.64	31.40	28.60	22.20	222
Grewia tilifolia	94.5	7.93	2.2	13.76	3.32	29.3	40.1	24.4	245
Grewia villosa	93.2	8.71	3.0	18.81	3.38	28.31	33.8	41.1	240

Graph No. 1:



Graph No. 2:



CONCLUSION:

From the preset investigation of *Grewia species* it revealed that these plants contain several pharmacognostic attributes and biochemical compounds known to be present and rich in proximate concentration. The present study will also be useful in the utilization of medicinal properties of these plant species and will enhance the further development of new herbal products and drug investigations. Further studies on pharmacognostic aspect and separation of many phytoconstituents will also be helpful in search of new chemical constituents. The work carried out concerning the phytochemical and pharmacological aspects of genus *Grewia* is not to the complete extent therefore novel work can be carried out in near future regarding the pharmaceutical, phytochemical, pharmacological and proximate aspects.

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