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Preliminary Phytochemical Investigation and comparative Quantitative Assessment of different extracts of *Cuscutareflexa, Murrayakoenigii* and *Vitex negundo*.

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Abstract

Aim: Preliminary Phytochemical Investigation and comparative Quantitative Assessment of different extracts of *Cuscutareflexa, Murrayakoenigii*and *Vitex negundo*.

Material and Methods: Plant material collected from the local region of Kangra, Himachal Pradesh. Extraction of plant material is done with the help of hot percolation with solvents of different polarity index. Percentage yield and phytochemical investigation of herbs were determined in different solvents.The total phenolic content and flavonoid content were determined by the Folincalcateaeu method and Aluminium chloride method respectively.

Results: The percentage yield was more in hydroalcoholic solvent as compared to petroleum ether, chloroform and aqueous extracts of plant material.The phytochemical investigation reveals the presence of alkaloids, glycosides, phenols, flavonoids, tannins, carbohydrates in the extracts. The TPC and TFC of each extract when compared, it was found that hydroalcoholic extract of each plant have more content as compared to petroleum ether, chloroform and water extracts of the plants.

Conclusion: The study concluded that the hydroalcoholic extracts of plants *Cuscutareflexa, Murrayakoeinigii* and *Vitex negundo* have majority of phytochemicals. The total phenolic and flavonoid content was more in hydroalcoholic extract of each plant as compared to petroleum ether, chloroform and water extract of the plants.

Keywords: Phytochemical screening, Total Phenolic content, Total Flavonoid content,*Cuscutareflexa, Murrayakoenigii, Vitex negundo*

Introduction:

Various disorders or ailments results due to tissue injury due to the release of various inflammatory mediators and lysosomal enzymes [1,2]. Inflammatory mediators and Reactive oxygen species are the major cause of inflammation from the activated neutrophils and macrophages [3]. Inflammation for a longer period leads to the development of various disorders like rheumatoid arthritis and various autoimmune disorder. Rheumatoid arthritis is a chronic inflammatory disease that results in joint pain and other skeletal disorders. According to WHO reports about 1 % of the world's population is suffering from arthritis. [4].About 70 % of the people living with rheumatoid arthritis are women and 55 % are older than 55 years. Rheumatoid arthritis is the most predominant disorder leading to numerous disabilities in human being, which limits the day-to-day activities [5,6]. The treatment of arthritis involves use of various NSAIDs and disease modifying antirheumatic drugs (DMARD's). These synthetic drugs used for the treatment of arthritis have been associated with various side effects and adverse effects. Although these drugs are also not cost effective.[7] Most of the medicines and food used in the modern era are based on the knowledge of traditional plants.In this modern society, plant-based food and medicines are used as a prime tool for sustaining the health and abetting in the retrieval of disease [8]. Arthritis can be treated with these traditional herbs from ancient times by our ancestors which did notshow any noticeable side effects.Traditional herbs are rich source of phenolic and flavonoid compounds which are major phytochemicals used in the treatment of various ailments and disorders.The herbs used in management of rheumatoid arthritis and can minimise the inflammatory disorders. Traditional herbs namely, *Vitex negundo* found in tropical and temperate climates including Asia, China, Indonesia, India. This shrub generally available in Himachal Pradesh and possess various pharmacological activities viz; anti-inflammatory, anti-leprotic, anti-arthritic, and many other properties [9,10].The second plant,*Cuscutareflexa* is a parasitic perennial herb that belongs to family Convolvulaceae. This plant found in South Asian countries like Pakistan, Nepal, India, Bagladesh. The plant is known to possess various pharmacological activities like anti-inflammatory, anticholinergic, anti-histaminic, anti-hypertensive activity [11]. The genus Murraya is rich in alkaloids, approximately 14 species of Murraya are available across the globe. Only two species of Murraya are available in India, out of which *Murrayakoenigii* belonging to family Rutaceae has been used in this study. Leaves of*Murrayakoenigii* are known to possess activities like antioxidant, hepatoprotective, anti-microbial, anti-fungal, anti-inflammatory and nephroprotective. Traditionally the leaves of

the plant have been used from ancient times as analgesics, digestives, appetizers as a home remedy. [12].

This present investigation involvesthe study of preliminary screening of these herbs in different solvents and estimation of total phenolic content and total flavonoid contentof these herbs in solvents of different polarity index.

Materials and Methods:

Collection of Plant Material: The plant material, whole plant of *Cuscutareflexa*, leaves of *Murrayakoenigii* and leaves of *Vitex negundo* were collected from the local region of Kangra, Himachal Pradesh in the month of September. The collected plant material was authenticated by botanist Dr. Madhava Chetty, Department of Botany, Shri Venkateswara University, Tirupati, Andhra Pradesh, India.The collected plant material washed in running water and then with distilled water to remove the dirt particles. Washed plant material then subjected to drying under shade for 90 hours. Dried plant materials were powdered into coarse powder with the help of an electric blender. The powdered plant material is then passed through sieve number 40 and kept in airtight containers for further use.[13]

Drugs and Chemicals: All reagents or chemicals used were of analytical grade. Folin-Ciocalteu (FC) Reagent, Aluminium chloride ($AlCl_3$), Sodium nitrite ($NaNO_2$), Sodium hydroxide (NaOH), anhydrous Sodium carbonate (Na_2CO_3), Sulphuric acid (H_2SO_4) and reference standards Gallic acid, Querecetinwere purchased from Merck and Sigma Aldrich. All the solvents, Petroleum ether, Chloroform, Acetone and ethanol used were of analytical grade. Rotary vacuum evaporator (Buchi Switzerland) was used for recovery of solvents under reduced pressure. UV/Vis Spectrophotometer (Shimadzu, India) was used for taking absorbance of test samples.

Extraction of Plant Material: The dried powdered plant material i. e. whole plant of *Cuscutareflexa*, leaves of *Murrayakoenigii*&*Vitex negundo*were extracted with different solvents. About 100 g of powdered material is subjected to Soxhlation with various organic solvents viz. Petroleum ether, chloroform, water, hydroalcoholic for the extraction of polar and non-polar organic compounds. The powdered material was first extracted with petroleum ether using Soxhlet apparatus (Borosil) for 72 hours at room temperature and then successively with chloroform, water and hydroethanolic solvent. Each solvent is subjected for extraction for 72 hours. After siphoning each extract is subjected to concentrate on a water bath and dried by using vacuum rotary evaporator. Then percentage yield of each

concentrated extract was calculated and stored in air tight containers in dessicator for further experimental evaluation.[14].

Preliminary Phytochemical Screening of Plant extracts: [13, 15]

The extracts were subjected to qualitative analysis for the presence of phytochemicals viz., carbohydrates, proteins, amino acids, steroids, glycosides, saponins, alkaloids, glycosides, tannins and flavonoids.

1) **Test for Carbohydrates:**
    - **Fehling's Test:** 1 ml Fehling's A and 1 ml of Fehling's B solutions were mixed, boil for one minute in test tube. Then equal volume of test solution added into it and heat in boiling water bath for 5-10 minutes. First yellow and then brick red ppt. was observed.
    - **Molisch Test:** To 2-3 ml of aqueous extract, few drops of α-napthol solution in alcohol were added,conc. Sulphuric acid added from the sides of the test tube. Violet ring is formed at the junction of two liquids.
2) **Test for Proteins and Amino acids:**
    - **Millon's Test:** To 3 ml of filtrate, 5 ml of millon's reagent added, white precipitate confirms the presence of proteins.
    - **Biuret Test:** To 3ml of test solution, few drops of 4 % sodium hydroxideand 1 % copper sulphate added, reaction mixture gives violet or pink colour.
3) **Test For Flavonoids:**
    - **Shinoda Test:** To dry powder or extract,5 ml 95% ethanol, few drops of concentrated hydrochloric acid and 0.5 g magnesium turnings added to the mixture in test tube, Orange, pink, red to purple colour appears.
4) **Test for Alkaloids:**
    - **Mayer's Test:** To 2-3 ml of filtrate, few drops of Mayer's reagent added to the reaction mixture and observe the presence of precipitate.
    - **Wagner's Test:** To 2-3 ml of filtrate, few drops of wagner's reagent added, gives reddish brown precipitate.
5) **Test For Phenols& Tannins:**
    - **Ferric chloride Test:**T 2-3ml of extract, few drops of 5 % ferric chloride solution added, reaction mixture will give deep blue-black colour.
6) **Test For Glycosides:**

- **Borntrager's Test:** To 3 ml of extract, dilute sulphuric acid added to the test tube. Boil and filter. To cold filtrate, equal volume benzene or chloroform. added and shake the mixture. Separate the organic solvent, ammonia added to the reaction mixture. Ammonical layer turns pink or red.

7) **Test for Terpenoids:**
   - **Salkowski reaction:** To 2 ml of extract dispersion, chloroform (2 ml) and concentrated sulphuric acid (2 ml) added with continuous shaking. Separation of chloroform layer and greenish yellow fluorescence in acidic layer for positive test of terpenoids.

8) Test for Saponins:
   - **Foam Test:**To each extract in a test tube, distilled water added with shaking and observe appearance of foam.

Determination of Total Phenolic Content:[16]

The total Phenolic Content was determined by the spectrophotometric method with the Folin-Ciocalteu Reagent according to the method explained by Stankovic et. al. (2012) with some modifications. The extracts of weight 50 mg were dissolved in 10ml of ethanol for making a stock solution. Each plant extracts of 0.5 ml were taken and mixed with the 0.5 ml of FC reagent (1:1). The reagent was diluted with the distilled water. The mixtureswere incubated for five minutes at 22 °C. After that 2 ml of 20% Sodium Carbonate added to all the above reaction mixture. Then the mixtures were incubated at 22 °C for one and half hour. The absorbance was measured at 650 nm. The samples were prepared in triplicate, and the mean value of absorbance was obtained. The same procedure was repeated for gallic acid as standard, and the calibration line was constructed. A set of gallic acid standard solutions (20, 40, 40, 60, 80 and 100µg/ml) were prepared.The content of total phenolic compound was expressed as mg of GAE/100gm of extract.

Determination of Total Flavonoid Content:[17]

The Total flavonoid content of differentextracts determined by Colorimetric method. Each sample 0.5 ml taken in 5 ml of flask and 2 ml of distilled water was added into the flask. Then 0.15 ml of 5% sodium nitrite added into the flask. The mixtures were incubated for 5 minutes at 25˚C. This was followed by addition of 0.3 ml of 10% $AlCl_3$ immediately. Then add 1 ml of 1 M Sodium hydroxide solution into it and then the mixture is diluted using distilled water upto 5 ml. A set of standard solutions of Quercetin of different concentrations (20,40,60,80 and 100 µg/ml) were prepared as mentioned above.The absorbance for test and

standard solution was measured at 510 nm wavelength using UV-Visible spectrophotometer. The total content of flavonoid was denoted as mg of QE/ 100g of extract.

Results and Discussion:

The collected plant material after solvent extraction were dried at room temperature. The obtained extracts after drying in Rotary evaporator and yield of different extracts were obtained. Maximum yield was obtained in the hydroalcoholic extract as compared to other solvents of each plant.The percentage yield of the aerial parts of the plant *Cuscutareflexa, Murrayakoenigii*and *Vitex negundo* in different solvents are tabulated in Table no. 1.

Table No. 1: The percentage yield of different extracts viz.; Petroleum ether, chloroform, aqueous and hydroalcoholic extract of whole plant of *Cuscutareflexa*, leaves of *Murrayakoenigii* and *Vitex negundo*.

Sr. No.	Name of Plant	Type of Extract	Weight of powdered Plant material (g)	Weight of crude extract (g)	%age yield of extract
1.	*Cuscutareflexa*	Petroleum ether	100	9.87	9.87
		Chloroform		10.07	10.07
		Aqueous		11.32	11.32
		Hydroalcoholic		19.81	19.91
2.	*Murrayakoenigii*	Petroleum ether	100	10.81	10.81
		Chloroform		7.80	7.80
		Aqueous		9.15	9.15
		Hydroalcoholic		17.81	17.81
3.	*Vitex negundo*	Petroleum ether	100	12.82	12.82
		Chloroform		11.50	11.50
		Aqueous		14.52	14.52
		Hydroalcoholic		22.55	22.55

Qualitative Phytochemical Analysis:

Phytochemical screening of different extracts of four different extracts viz; petroleum ether, chloroform, aqueous and hydroalcoholic extract of whole plant of *Cuscutareflexa*, leaves of *Murrayakoenigii*and *Vitex negundo* showed the presence of phytochemicals like

carbohydrates, proteins, tannins, flavonoids, phenols, alkaloids and glycosides. The majority phytochemicals are present in hydroalcoholic extract of *Cuscutareflexa, Murrayakoenigii* and *Vitex negundo*. Presence of secondary metabolites in different solvents of aerial parts of the plants of*Cuscutareflexa, Murrayakoenigii* and *Vitex negundo* shown in the Table no. 2.

Table 2: Table showing Preliminary Phytochemical Screening of different extracts viz.; Petroleum ether, chloroform, aqueous and hydroalcoholic extract of whole plant of *Cuscutareflexa*, leaves of *Murrayakoenigii* and *Vitex negundo*.

Phytochemical	**Test Name**	**Petroleum Ether**			**Chloroform**			**Aqueous**			**Hydroalcoholic**		
		C. r.	*M. k.*	*V. n.*	*C. r.*	*M. k.*	*V. n.*	*C. r.*	*M. k.*	*V. n.*	*C. r.*	*M. k.*	*V. n.*
Carbohydrates	**Fehling's Test**	-	-	-	-	-	-		-	+	-	-	+
	Molisch Test	-	-	-	-	-	-		-	+	-	+	+
Proteins	**Biuret Test**	-	+	-	-	+	-		+	+	-	+	+
	Millon's Test	-	+	-	-	+	-		+	+	+	-	+
Tannins	**Lead Acetate Test**	+	_	+	+	+	-	+	-	-	+	+	+
	Ferric Chloride Test	+	-	+	+	+	-	+	-	-	+	+	+
Flavonoids	**Aluminium Chloride Test**	+	+	+	+	+	+	+	+	+	++	+	++
Alkaloids	**Mayer's Test**	_	+	-_	-	+	++	+	-	-	+	+	++
	Wagner's Test	-	+	_	-	+	++	+	-	-	+	+	++
Phenol	**Ferric Chloride Test**	-	+	+	+	-	++		-		+	+	++

Glycosides	**Borntrager's Test**	+	_	-	+	_	_	_	-	+	+		
Terpenoids	**Salkowski Test**	+	+	+	+	-	_	+	-	-	+	+	+
Saponins	**Foam Test**	-	+	-	-	+	-	+	+	-	+	+	-

(+) sign indicates presence of phytochemical, (-) sign indicates absence of phytochemical. (Abbreviations;*C.r.: Cuscutareflexa, M.k.: Murrayakoenigii, V.n.: Vitex Negundo*)

Estimation of Phytoconstituents:

The total phenolic content and total flavonoid content of aerial parts of the plant*Cuscutareflexa, Murrayakoenigii*and *Vitex negundo* of different polarity index shown in Table no. 3and 4.

Table 3: Total Phenolic Content of different extracts of *Cuscutareflexa, Murrayakoenigii* and *Vitex negundo*

Sr. No.	**Plant Name**	**Total Phenolic Content (mg/100gm of dried extract)**			
		Pet. Ether extract	**Chloroform extract**	**Aqueous Extract**	**Hydroalcoholic Extract**
1.	*Cuscutareflexa*	90.23±0.04	101.25± 0.01	124.74±0.07	252 .82±0.08
2.	*Murrayakoenigii*	102.42± 0.09	95.65±0.08	115.78±0.05	274.75±0.04
3.	*Vitex negundo*	95.43± 0.07	109.4±0.09	131.45±0.02	236.01±0.07

Total Phenolic Content: Results expressed as mg of gallic acid equivalent/ 100 gm of crudeextracts; Each value in the table is the mean (±) standard deviation (n=3)

Table 4: Total Flavonoid Content of different extracts of *Cuscutareflexa, Murrayakoenigii* and *Vitex negundo*

Sr. No.	**Plant Name**	**Total Flavonoid Content (mg/100gm of dried extract)**			
		Pet. Ether extract	Chloroform extract	Aqueous Extract	Hydroalcoholic Extract
1.	*Cuscutareflexa*	80.12±0.18	95.78±0.04	116.18±0.01	264.15±0.09

2.	*Murrayakoenigii*	98.21±0.03	100.02±0.06	129.03±0.02	773.05±0.07
3.	*Vitex negundo*	95.32±0.02	98.56±0.20	139.56±0.06	502.41±0.08

Total Flavonoid Content: Results expressed as mg of quercetin equivalent/ 100 gm of crude extracts; Each value in the table is the mean (±) standard deviation (n=3)

The content of total phenols of different extracts of plants *Cucuta reflexa*, *Murrayakoenigii*and *Vitex negundo* in different polarity index was evaluated by FCR method using gallic acid as standard as shown in Figure no. 1 and absorbance values in Table no. 4.The content of total phenols was found maximum in hydroalcoholic extract of *Murrayakoenigii*is 274.75 mg /100 gm. Figure no. 3 shows the comparison of total phenolic content of different extracts.

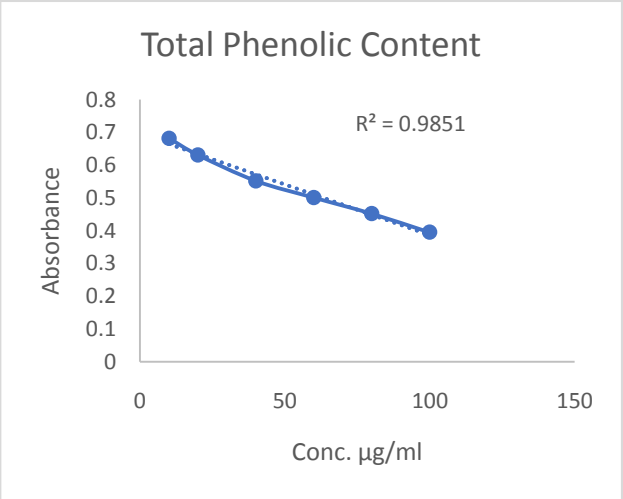

Figure No. 1: Gallic acid Standard curve of absorption against gallic acid concentration; R ^{2}=0.9851

Table no. 4: Table showing o.d. values of gallic acid standard at different concentration

S No.	**Concentration in mcg**	**Absorbance**
1	10	0.62
2	20	0.631
3	40	0.552
4	60	0.501
5	80	0.452
6.	100	0.395

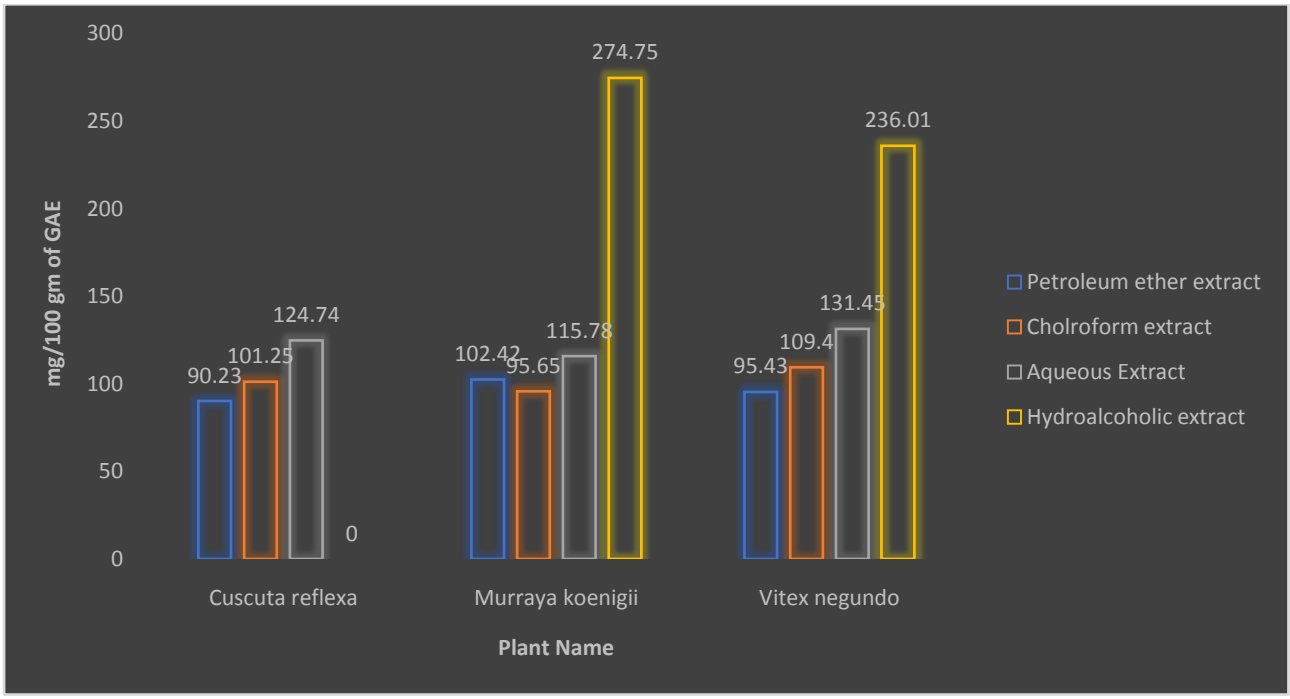

Figure No. 2: Comparison of Total phenolic Content of different extracts of *Cuscutareflexa, Murrayakoenigii* and *Vitex negundo* in different solvents

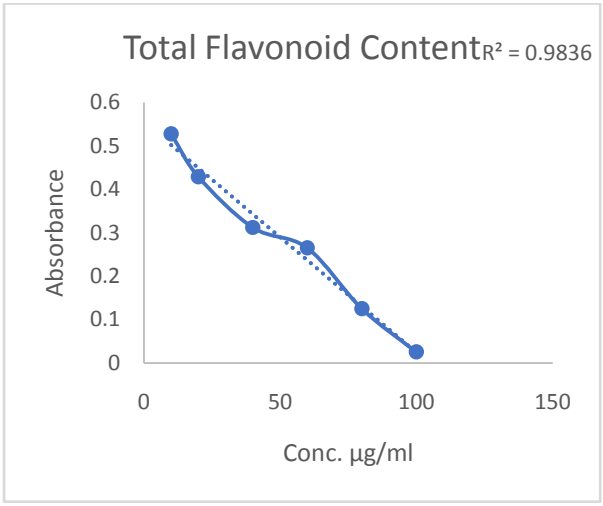

Figure No. 3: Standard curve of absorption against quercetin concentration; R ^{2}=0.9836

Table no. 5: Table showing o.d. values of Quercetin standard at different concentration

S.No.	Concentration	Absorbance
1	10	0.528
2	20	0.429
3	40	0.312
4	60	0.265
5	80	0.125
6.	100	0.025

The content of total flavonoid of different extracts of plants *Cucuta reflexa*, *Murrayakoenigii*and *Vitex negundo* in different polarity index was evaluated by Aluminium chloride method using quercetin as standard as shown in standard curve Figure no. 3 and absorbance value in Table no. 5.The content of total flavonoid was found maximum in hydroalcoholic extract of *Murrayakoenigii*is 773.05 mg /100 gm. Figure no. 4 shows the comparison of Total flavonoid content of different extracts of plants.

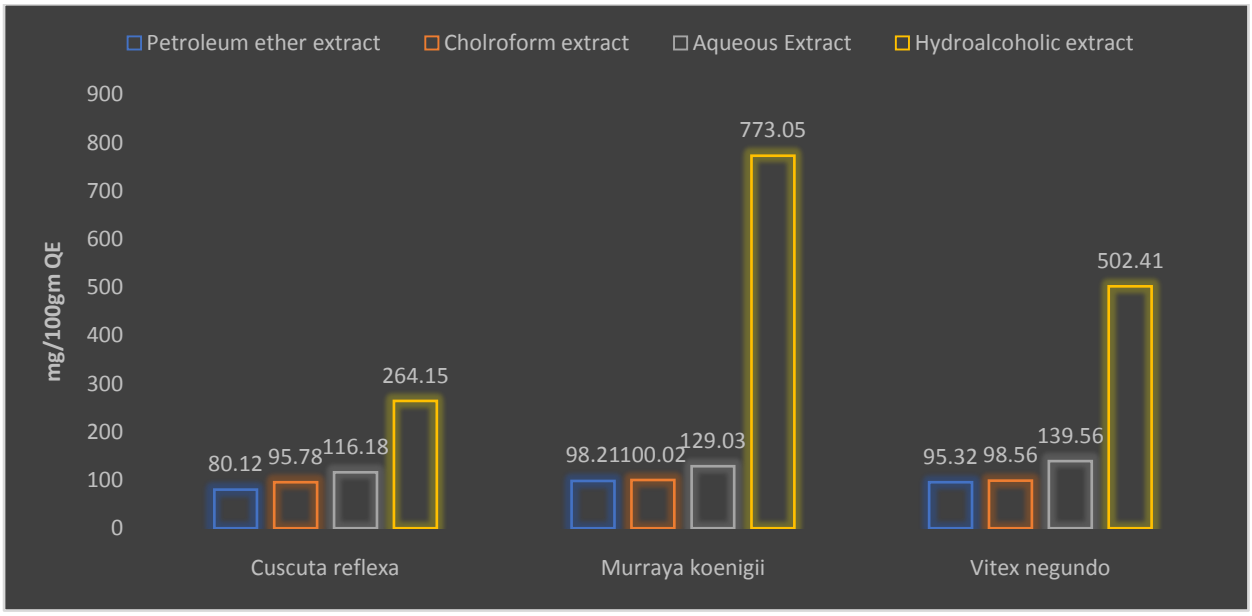

Figure No. 4: Comparison of Total Flavonoid Content of different extracts of *Cuscutareflexa, Murrayakoenigii*and *Vitex negundo* in different solvents.

Discussion:

Preliminary phytochemical screening of herbal extracts of different polarities suggests the presence of various phytochemicals like alkaloids, glycosides, tannins, phenols, flavonoids, and saponins. The majority of phytochemicals were detected in the hydroalcoholic extract of different plants. The total phenolic and flavonoid content of different extracts of *Cuscutareflexa*, *Murrayakoenigii* and *Vitex negundo* were different. A gallic acid standard calibration curve was used for the determination of amount of total phenolic content with R^2=0.9851 value. The maximum number of total phenols in *Cuscutareflexa* extract were in the order; Hydroalcoholic extract> aqueous extract> chloroform extract> Petroleum ether extract, in *Murrayakoenigii*extract viz; Hydroalcoholic extract> aqueous extract> Petroleum ether extract> chloroform extract and in *Vitex negundo* extract the order of phenolic content; Hydroalcoholic extract> aqueous extract> chloroform extract> Petroleum ether extract.A quercetin standard calibration curve was used for the determination of amount of total flavonoid content with R^2=0.9836 value. The maximum number of total flavonoids in plant *Cuscutareflexa*, Murrayakoenigii and Vitex negundo were in the order viz; Hydroalcoholic extract> Aqueous extract> Chloroform extract> Petroleum ether extract. This finding suggests that the maximum content of total phenols and flavonoids were maximum in hydroalcoholic extract of each plant as compared to the other solvents.

Conclusion:

This study suggests that three herbs indicate the presence of phytochemicals in different extracts. Quantitative estimation of extracts showed that the hydroalcoholic extract of each plant have more content than another extracts. Phenols and flavonoids are the potential which are used in the treatment of various ailments through different mechanisms. So, hydroalcoholic extract of each plant can be taken as potential in future for the management of inflammation and other inflammatory disorders like rheumatoid arthritis and autoimmune disorders.

Conflict of Interest: No Conflict of interest.

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