CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1304293

Available online at: http://www.iajps.com Research Article

PHYTOCHEMICAL AND PHYSICOCHEMICAL ANALYSIS OF CONVENTIONAL AND MICROWAVE ASSISTED EXTRACTION OF *VITEX NEGUNDO LINN* LEAVES

Samreen Fatema[1], Mazahar Farooqui[2], Sunil Jadhav[1-2] and Pathan Mohd Arif*[1]

[1]Post Graduate and Research Center, Maulana Azad College, Aurangabad (MS), India 431001.

[2]Dr. Rafiq Zakaria College for women, Navkhanda, Aurangabad (MS) India 431001.

Abstract:

Vitex negundo Linn commonly known as five leaved chaste tree in English, Nirgudi in Marathi, nirgundi in hindi and indrani in Sanskrit, which have great medicinal value. The present study was done developed extraction method. Microwave extraction and conventional extraction was carried out and both the extracts were compared with their percent of extraction, phytochemical constituent, physicochemical properties and by UV-Vis spectra. Four new parameters were introduced that is bulk density, tapped density, carr's index and housner's ratio which was previously not reported. It is observed that microwave and conventional extraction gives different results. This may be due to difference in energy supply for both the extract.

Keywords: *Vitex nirgundo, Phytochemical, Physicochemical, Microwave extraction, Bulk density, tapped density.*

Corresponding author:

Pathan Mohd Arif,

Post Graduate and Research Center,
Maulana Azad College,
Aurangabad (MS), India 431001.
Email: arif7172@rediffmail.com
Mobile: +919423456007

QR code

Please cite this article in press Pathan Mohd Arif et al., ***Phytochemical and Physicochemical Analysis of Conventional and Microwave Assisted Extraction of Vitex Negundo Linn Leaves,*** *Indo Am. J. P. Sci, 2018; 05(06).*

INTRODUCTION:

Herbal medicines are used for the treatment of many infectious diseases from ancient time. Plants play an important role in primary health care. The investigation of medicinal plants in different parts of the world is important for both the sectors agriculture and medicines [1]. *Vitex negundo Linn* belongs to the family *Verbenaceae* is commonly known as five leaved chaste tree in English, nirgudi in matrathi, nirgundi in hindi, indrani in Sanskrit. This is large aromatic slender tree, growing up to 4.5 m in height. Stems covered by thin gray bark, which converted in black color as it gets old. This plant occurs in most part of India near moist places [2]. This plant is useful and used to treat Osteoporosi Bone mineral density characterizely decrease, the risk of fractures increases and it is related with micro architectural deterioration of bone tissue, is called as osteoporosi. Reduced estrogen level can cause osteoporosis which is common in women. The risk for the coronary heart disease and osteoporosis can be lowered by hormone replacement therapy (HRT) but it increases the risk of certain types of cancer like endometrial and breast cancer [3] Samuel et al shows that the hexane, diethyl ether and ethyl acetate extract of *Vitex negundo* has larvicidal activity [4]. Water extract of *Vitex negundo* used as anti-inflammatory, analgesic and anti-itching agents internally and externally in the Ayurveda remedies. M.G. Dharmasiri et al shows investigate that mature fresh leaves of *Vitex negundo* has dose-dependent activity against inflammation [5]. Anti-convulsant and anti-oxidant properties [6]. Vishal Tandon et al reported that the ethanol extract of *Vitex negundo* leaves possess hepatoprotective activity against the hepatotoxicity [7]. Keeping in view above facts we studied the Phytochemical constituents by conventional and microwave extraction method.

MATERIAL AND METHODS:

All the chemicals used for the present study was of anal R grade and of S.D fine chemicals Ltd. For the qualitative test reagents are prepared in double distilled water that was prepared by first distilled using metal distillation assembly and then by using quick fit glass assembly. For the determination of physicochemical parameters care was taken to use pure solvents. The leaves sample of *Vitex negundo Linn* plants were collected from nearby area of Vasantrao Naik college Cidco, Aurangabad. The leaves were washed properly and dried under shade for 6 days. The dried leaves were grind by using kitchen grinder to fine powder.

Fluorescent test:

0.5 gm of sample was added in different solvents and fluorescent behavior was observed by naked eye.

Ash analysis:

The procedure is used for the determination of ash analysis is followed according with the Ayurvedic Pharmacopeia. Accurately weighted 10 gm sample was taken in finely clean silica crucible and ignite for 4 hrs with gradually increasing temperature up to 300^{o}C. After ignition of leaves of plant, the residue remains is designated as ash. The residue was again ignited with the interval of 10 min, till we get the constant weight. This ash was used to determine the three parameters i.e as total ash, acid insoluble ash and water soluble ash.

Acid insoluble ash: Portion of ash which is insoluble in dilute HCl. 1 gm of total ash was dissolved in 2 N hydrochloric acid, stirred well for the digestion and filtered through wattman filter paper no. 41. The residue remains after filtration is ignited in clean silica crucible by gradually increasing temperature up to 300^{o}C. The residue was cooled, weighed and again kept for ignition till to get the constant weight. The residue is remain after ignition is acid insoluble ash. The percentage of acid soluble ash was calculated.

Water soluble ash: 1 gm of total ash was boiled with20ml of double distilled water. The residue was collected by filter through wattman filter paper no 41. Residue was washed with hot water and kept for ignition not more than 400^{o}C. The weight of residue was subtracted from total ash. This difference between residue and total ash represent the water soluble ash. The percentage of water soluble ash was calculated.

Bulk density: 50 cm^3 of powder was introduced into the 50 ml graduated cylinder. The dropping interval of the cylinder was two sec at the height of 2 cm three times on the hard wooden surface. The bulk density was calculated by dividing the weight of the sample in grams by the final volume in cm^3 of the sample contained in the cylinder.

Tab density: 50 cm^3 of powder was introduced into the 50 ml graduated cylinder. The dropping interval of the cylinder was two sec at the height of 2 cm 100 times on the hard wooden surface. The tab density was calculated by dividing the weight of the sample in grams by the final volume in cm^3 of the sample contained in the cylinder.

The compressibility of the powder was evaluated using the HR (Housner Ratio). The Housner ratio may be defined as ratio of tap density and bulk density.

$$HR=\frac{TD}{BD}$$

Carr's Index (Compressibility Index- CI): This was calculated by using the formula:

$$CI = \frac{Dt - Db}{Dt} X\ 100$$

Extraction procedure:

Water extraction: Accurately weight 30 gms of sample was introduced into the 500 ml round bottom flask (which was first clean by very dilute hydrochloric acid and then distilled water) with 300 ml double distilled water. The pourciline pieces were add to avoid bumping of the sample. The condencer was fitted with circulation of water. The sample was refluxed on flame for six hours. The sample was cooled and filtered by the suction pump. The excessive water was evaporated for the preservation of the sample and it was kept at 4°C for 12 hrs. The percentage of the extract was calculated.

Microwave extraction: 30 gms of the sample was kept in the clean round bottom flask. 300ml of double distilled water was used as the solvent. The porciline pieces were added to avoid bumping of the sample. The condenser was fitted with circulation of water. The sample was refluxed by microwave radiations using microwave oven.

Physico chemical test:

Relative density: clean and dry empty density bottle with stopper weighted accurately .The density bottle was filled with double distilled water up to it fall from the bottle and stopper was fitted and the bottle was cleaned from outside. The bottle was weighted.
The procedure was repeated for the samples. The density was measured by taking difference between bottle with sample and empty bottle. Relative density was calculated by the formula

$$\text{Relative density} = \frac{\rho_2}{\rho_1}$$

Where ρ_1 is density of the water and ρ_2 is the density of the sample.

Viscosity: The different concentration in ppm of the samples were prepared by using double distilled water. The Ostwald's viscometer was cleaned by NaOH to remove greasy impurities than with chromic acid and finally with the distilled water. The 10 ml of double distilled water was inserted in viscometer from large diameter tube. And the sample was sucked through second tube of the same viscometer till it rises with 2-3 cm above the mark. By keeping stop watch ready the liquid was allowed to decent down the time required to flow of the liquid between two points was noted. The same procedure was repeated for the samples which have to study.
Viscosity was measured by sing formula

$$\eta_2 = \frac{t_2}{t_1}\frac{\rho_2}{\rho_1}\eta_1$$

Surface tension: The different concentration in ppm of the samples were prepared by using double distilled water. The stalagmometer was cleaned by NaOH to remove greasy impurities than with chromic acid and finally with the distilled water. The rubber tubing with the with a screw clip was attached to the top of the stalgmometer. The flat end of the stalagmometer was dipped into the standard solution (double distilled water) suck through the water tubing until the water level rises above the mark. The screw was adjust the pressure until the rate of the drop was 10 to 15 per minute. The number of drops were counted for double distilled water when passes from upper mark to the lower mark.

The stalagmometer was removed and rinse with alcohol and dried. Stalagmometer was filled with the test sample and number of drops were determine. Same procedure was repeated for every concentration three times and mean was taken.
The surface tension was than calculated by the formula

$$\gamma_2 = \frac{n_1}{n_2}\frac{\rho_2}{\rho_1}\gamma_1$$

Where γ_1 and γ_2 are the surface tension of the double distilled water and the sample respectively.
And n_1, ρ_1 and n_2, ρ_2 are the number of drops and relative densities of the double distilled water and samples under study respectively.

Refractive index Refractive index depends upon temperature and concentration. However the specific refraction is independent of temperature and it is characteristic property of the liquid. The refractive index was measured by Abbe's refractometer. The refractometer was placed on the table near to the window so that sufficient light should reached to the prism. The prism box was opened b turning the lock nut and both the phases of the prism was cleaned with the help of cotton wool by the acetone and prism box was closed after drying. Few drops of the liquid were pumped through the small hole on the prism box with the help of the dropper. The crosswire of the telescope was focus by rotating the eye piece and the mirror was adjust for the reflection of maximum light towards the prism box. The prism box was moved forward and backward until the clear boundary between the light and dark region was appear. The scale reading was noted down. And refractive index were calculated by using the formula

$$\text{Specific refraction (r)} = \frac{n^2-1}{n^2+2} \mathbf{X} \frac{1}{\rho}$$

Where ρ is the density of the samples.

Qualitative Phytochemical Screening:
The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative Phytochemical analysis was done using the following procedures.
Detection of alkaloids: Solvent free extract, 50 mg was stirred with 5 mL of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.
i) Mayer's test: To a few mL of filtrate, two drop of Mayer's reagent added by the side of test tube. A white or creamy precipitate indicated the test as positive.
Mayer's reagent: Mercuric chloride (1.36 g) was dissolved in 60 mL of water and potassium iodide (5.0 g) was dissolved in 10 mL of water. The two solutions were mixed and made up to 100 mL with water.
ii) Wagner's test: To a few ml of filtrate, few drops of Wager's reagents were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.
Wagner's reagent: Iodine (1.27 g) and potassium iodide (2 g) was dissolved in 5 ml of water and made up to the 100 ml with distilled water**.**
iii) Hager's test: To a few ml of filtrate 1 or 2 of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the test as positive.

Detection of Carbohydrate:
The extract 100 mg was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.
i) Molisch's tests: To 2 ml of filtrate, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of test tube and allwed to stand. A violet ring indicates the presence of carbohydrates.
ii) Fehling's test: One ml of filtrate was boiled on water bath with 1 ml each Fehling's solutions A and B. Red precipitate indicates the presence of suger.
Fehling's solution: Solution A; copper sulphate (34.66 g) was dissolved in distilled water made up to 500 ml with distilled water.
Solution B: potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made up to 500 ml.
iii) Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristics colored precipitate indicates the presence of sugar.
Benedict's reagent: Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml distilled water and boiled to make it clear solution. Copper sulphate (17.3 g) dissolved in 100 ml distilled water.
iv) Barfoed's test: To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 min. red precipitate indicates the presence of suger.
Barfoed's reagents: copper acetate 30.5 g was dissolved in 1.8 ml of glacial acetic acid.
Detection of glycosides: For the detection of glycosides, 50 mg of extract was hydrolysed with concentrated hydrochloric acid for 2 hrs on water bath, filtered and the hydrolysate was subjected to the following test.
i) Borntrager's test: To 2 ml of the filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was seprated and 10% ammonia solution was added to it. Pink color indicates the presence of glycosides.
ii) Legal's test: Fifty mg of extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycoside was indicated the pink color**.**
Detection of Saponins by Foam test: The 50 mg was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

Detection of proteins and Amino acids:
The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through Whatman filter paper no. 41 and filtrate was subjected to test of proteins and amino acids.
i) Millon's test: To 2 mL of filtrate, few drops of Millon's reagent were added. A white precipitate indicated the presence of proteins.
Millon's reagent: Mercury (1 g) was dissolved in 9 mL of fuming nitric acid. When the reaction was completed, equal volume of distilled water was added.
ii) Biuret test: An aliqot of 2 ml of filtrate was treated withone drop of 2 % copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicates the presence of proteins.
iii) Ninhydrine test: two drops of ninhydrine solution (10 mg of ninhydrine in 200 ml of acetone) were added to two ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of phenolic compounds and tannins:

i) Ferric chloride test: The extract (50 mg) was dissolved in 5 ml distilled water. To this few drops of neutral 5% ferric chloride solutions were added. A dark green color indicates the presence of phenolic compounds.

ii) Gelatin test: The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% solutions of gelatin containing 10% sodium chloride was added to it. White precipitate indicates the presence of tannins.

iii) Lead acetate tests: the extract (50 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of flavonoids compounds.

iv) Alkaline reagent test: an aqueous solution of the extract was treated with the 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Spectral analysis: UV-Visible: The powder of extract was dried and dissolved in distilled water to prepared solution of approximately 50 ppm. The spectra was recorded in the range from 190 to 800 nm by using double beam spectrophotometer of Model Elico-159 and λmax is determine from the software Spectra treat**.**

IR Spectra: The FTIR instrument IRT3000, JASCO, having serial no. B051061016, and the spectra were recorded using spectra manager. IR instrument is calibrated by using polystyrene. Spectra were recorded by using potassium bromide (KBr) of IR grade manufactured by Marck life sciences. KBr was kept in hot oven at 50^{o}C for half hour to free it from moisture. Spectra of that dry KBr is measured within IR range. The samples of leave extract were crushed to make it fine powder and mixed with dry KBr, and spectra of the samples were measured.

RESULT AND DISCUSSION:

Fluorescence study: The fluorescence study of *feronia limonia* showed by M.K Gupta [8]. The powdered samples were treated with different chemicals and observed with naked eyes. The various chemical constituents present in the plant material may exhibit fluorescence phenomena. Sometimes when treated with different reagents non-fluorescence may convert into fluorescence and emits characteristics radiations in day light. Normal light florescent behavior was different in different solvent. This is used for the qualitative assessment of the plant material [9]. The result of fluorescent test were summarized in table 1: It is observed that the plant leave powder show different color in strong mineral acids, while in dilute acid there is no change in colour. This may e attributed to strength of acid which causes either decomposition or oxidation of plant leaves. The change in color in basic media is due presence of phenolic group.

Table 1: Florescent test for leave powder of *Vitex negundo*

Sr. no.	Solutions	Observation
1	Powder as such (P)	Dark green
2	P + n-butanol	No change
3	P + Conc. HCl	Reddish yellow
4	P + Conc. HNO_3	Dark orange
5	P + Conc. H_2SO_4	Blackish brown
6	P + Chloroform	No change
7	P + Ethanol	Cream
8	P + Glacial acetic acid	No change
9	P + 1N HCl	No change
10	P +1N NaOH	Dark yellow
11	P + 5% HCl	No change
12	P + 5% NaOH	Dark yellow
13	P + benzene	No change

Ash analysis: The total ash content of the powder is found to be 8.3%. The acid insoluble ash was found to be 35%. Whereas water soluble as 45 %. The ash is mostly consist of transition elements i.e. cobalt or magnesium. The tab density and bulk density of the powdered material is given in table 2.

Table 2: Ash analysis and densities of leave powder

Sr. No.	Ash	Results
1.	Total ash	8.3%
2.	Acid insoluble ash	35%
3.	Water soluble ash	45%
4.	Bulk density	0.4333
5.	Tab density	0.4980
6.	Houser ratio	1.150
7.	Carr's ratio	13.05

Percentage extraction: The different chemical constituents are present in different extract from same sample is due to solubility, solvent used and method of extraction[10,11]. The traditional extraction process posses various advantages and disadvantages. Water is a safe solvent for the extraction, but different Phytochemical shows variable degree of extent of solubility. The organic solvent has few disadvantages. There is increasing public awareness of safety hazards associated with the use of organic solvents in herbal processing and the possible solvent contamination of the final product [12]. In the present study leaves of *Vitex negundo Linn* is subjected for the extraction using same solvent but different methods of extraction that is conventional extraction (CE) and microwave assisted extraction (MAE). It is found that MAE is more superior as compared to the CE. MAE was done for 30 min at 60% power, 420 watt and 120^{o}C. The sample was cooled and filtered by the suction pump. The excessive water was evaporated for the preservation of the sample and it was kept at 4^{o}C for 12 hrs. The percentage of the extract was calculated. The percentage is summarized in table 3.

Table 3: Extractive value of *Vitex negundo Linn* plant leaves.

Sr. No.	Solvent	Percentage
1.	Conventional Hydro Distillation	20.65
2.	Microwave Assisted Hydro Distillation	17.86

Phytochemical test: The standard herbal drug may get contaminated by foreign organic matter or may be replaced or adultrated by low quality crude drugs. Hence it is necessary to have phrmacognestic and Phytochemicals profile of plant [13]. The medicinal plants exhibit anti-diarrhoeal properties due to tannins, alkaloids saponins, flavonoids, sterols and reducing agent [14]. The water extract may have a low concentration of antibacterial components or the components may not have been completely extracted in water [15]. The K. Sahayaraj has done the phytochemical study of chloroform, benzene and water extract of vitex nirgundo leaves. Water and chloroform extract contain more phyto-ingrediants, from that also only water extract gives positive test for Xanth proteins and alkaloids, while it is absent in chloroform and benzene [16]. The presence of phytochemicals in the *Vitex negundo Linn* is summarized in table 4

Table 4: Qualitative test for *Vitex negundo Linn* leaves extract

Sr. No.	Reagent	CHE	MAHE
1.	**Detection of Alkaloids**		
A.	Mayer's test	-ve	-ve
B.	Wagner's test	-ve	-ve
C.	Hager's test	-ve	-ve
2.	**Detection of carbohydrate**		
A.	Molish test	+ve	+ve
B.	Fehling's test	+ve	+ve
C.	Benedic test	-ve	-ve
D.	Barfoad's test	+ve	+ve
3.	**Detection of Glycosides**		
A.	Borntrager's test	-ve	-ve
B.	Legal's test	-ve	-ve
4.	**Foam test**	+ve	+ve
5.	**Detection of proteins and amino acid**		
A.	Millon's test	+ve	-ve
B.	Nitric acid test	+ve	+ve
C.	Biuret test	+ve	+ve
D.	Ninhydrine test	-ve	-ve
6.	**Detection of phenolic compound and tannins**		
A.	Ferricchlorid test	+ve	+ve
B.	Gelatin tests	-ve	-ve
C.	Lead acetate test	+ve	+ve
D.	Alkaline reagent test	+ve	+ve

Physicochemical properties: Through, there are several sophisticated hyphenated techniques available for plant identification still physico-chemical study of plant drug is more reliable, accurate and inexpensive [17]. Physicochemical parameters determine were Relative density, viscosity, surface tension, Refractive index in table 5 and 6. The relative density and surface tension of CE was found to be higher. The refractive index was found to be less in MAE. The surface tension for the MAE found to be approximately constant over the range of concentration.

Table 5: Physicochemical properties of CE of *Vitex negundo Linn* Leaves extract.

Sr. no.	Solution in ppm	Relative density	Viscosity (Pascal sec)	Surface tension (Newton/meter)	Refractive index
1.	5	0.9963	0.86413	66.7977	0.99374
2.	10	0.9956	0.86671	71.9621	1.00192
3.	20	0.9967	0.83301	81.0472	1.00076
4.	40	0.9979	0.86871	81.1444	0.99956
5.	60	0.9967	0.86767	84.5701	1.00089
6.	80	0.9986	0.86932	74.9576	0.99899
7.	100	0.9996	0.87020	72.2535	0.99799

Table 6: Physicochemical properties of MAE of *Vitex negundo Linn leaves extract.*

Sr. no.	Solution in ppm	Relative density	Viscosity (Pascal sec)	Surface tension (Newton/meter)	Refractive index
1.	5	1.0038	0.8766	70.1779	0.9938
2.	10	1.0039	0.9122	70.1849	0.9937
3.	20	1.0019	0.8749	56.0339	0.9957
4.	40	1.0027	0.8757	57.7213	0.9949
5.	60	1.0007	0.8739	57.6071	0.9988
6.	80	1.0021	0.8752	56.0451	0.9935
7.	100	1.0035	0.8763	56.1217	0.9967

3.6 Spectral analysis: UV- spectrum: The maximum absorption peaks i.e λmax was observed at 199 and 197 nm for conventional and microwave assisted extract respectively. The spectrum pattern for aqueous extract and microwave assisted extract is found to be somewhat similar. UV-Vis spectra of both the extracts are shown in fig 6 & 7.

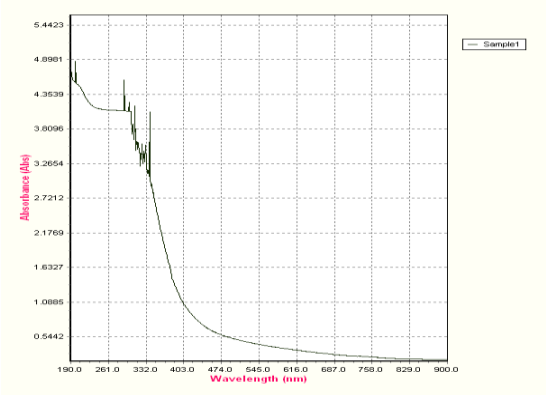

Fig 6: UV-visible spectra of CHE of *Vitex negundo Linn* plant leaves

Fig 7: UV-visible spectra of MAHE of *Vitex negundo Linn* plant leaves

FTIR spectrum:

The IR spectrum of extract of *Vitex negundo Linn* was recorded from SAIF Chandigarh. (fig1) Though it contains a mixture of compounds but still in order to find out various functional groups and a general finger print of samples, it will help. There are various IR bands observed. Which are represented in table 7.

Table 7. IR bands of *Vitex negundo Linn* plant leaves extract

Band (cm^{-1})	Intensity	Functional group
3348	Broad band	O-H, N-H
2919	Sharp	Salts of primary amines, cyclic alkane
1606	Sharp	C=C, C=N, N=O
1417	Broad	C-H bending vibrations
1075	Sharp	C-O-C asymmetrical stretching
818	Sharp	Fermiresonance bonding band between C=O
770	Sharp	Four adjacent hydrogen.

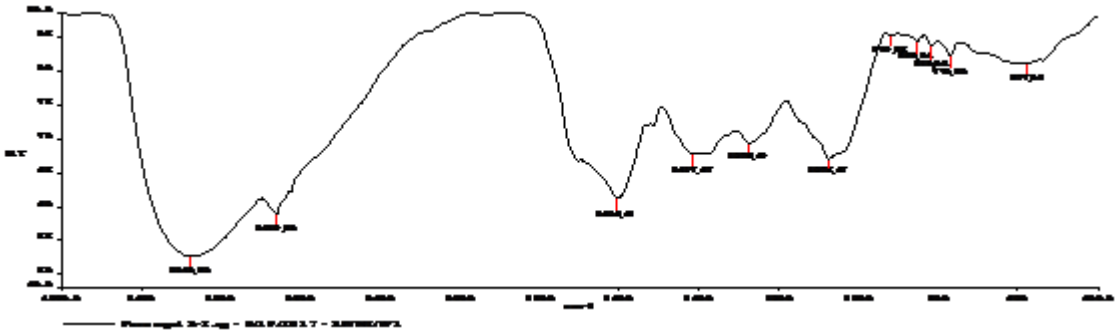

Fig 5: IR spectrum of *Vitex negundo*

CONCLUSION:

In the method of conventional extraction and microwave assisted extraction, microwave gives less percent of extraction as compared to the conventional extraction. But this percent of extraction can be increased by increase in time of extraction few minutes. MAE is more superior than CE because it gives near about same percentage with in short time. Both the extract gives same result for phytochemical analysis but it possess different physicochemical properties. IR was carried out for the investigation of the possible functional group. CE possess antibacterial activity while MAE shows negative results for all.

REFERENCES:

1. Zaidan M.R.S, Noor Rain A, Badrul A.R, Adin A Norazah A and Zakiah I, " In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method " tropical biomedicine, 2005, 22 (2), 165-170.
2. Kishor Vasant Otari, Om Gangadhar Bichewar, Rajkumar Virbhadrappa Shete, Chandrakant Devidas Upasani, " Effect of hydroalcoholic extract of *Vitex negundo Linn*, leaves on learning and memory in normal and congnitive deficit mice" Asian pacific journal of tropical biomedicine, 2012, s104-s111.
3. Manmeet Kumar, Preeti Rawat, Devendra Mishra, Abnish K. Gautam, Rashmi Pandey, Divya Singh, Naibedya Chattopadhyay, Rakesh Maurya, "Anti-osteoporoti c constituents from Indian medicinal plants" Phytomedicin, XXX(2010).
4. Samuel Tennyson, K John Ravindran, S. Arivoli, "Screening of twenty five plant extracts for Iarvicidal activity against Culex quinquefasciatus say (Diptera: cu;icidae)" Asian pacific journal of tropical biomedicine ,2012, s1130-s1134.
5. M.G. Dharmasiri, J.R.A.C Jayakody, G. Galhena, S.S.P Liyanage, W.D. Ratnasooriya, " Anti-inflammatory and analgesic activities of nature fresh leaves of *Vitex negundo*" Journal of ethanopharmacology, 2003, 87, 199-206.
6. Ram Chandra Dhakal, Meena Rajbhandari, Surya K. Kalauni, Suresh Awale and Mohan B. Gewali, "Phytochemical constituents of the bark of *Vitex negundo* L" journal of Nepal chemical society, vol (23), 2008/2009, 89-92.
7. Vishal R Tandon, V. Khajuria, B. Kapoor, D. Kour, S. Gupta, " Hepatoprotective activity of *Vitex negundo* leaf extract against anti-tubercular drugs induced hepatotoxicity" Fitoterapia, Elsevier, 79(2008), 533-538.
8. M K Gupta, Ramesh Kumar Singh, " preliminary pharmacognostical investigation of feronia limonia leaves" IJPI's journal of pharmacognosy and herbal formulation. 2 (7), 2011, 1-5.
9. M Suja, Suyambu Rajan, Thiyagrajan Thirunalasundari, Brinda Jana and Sambadam Thennozhi, "pharmacognistical and Phytochemical studies of an ayurvedic drug *Saraca asoca* stem bark" , journal of pharmacy research, 5(2), 2011, 1119-1121.
10. Arvind Kumar, Sadhana singh, Kanhiya Mahour, V.S Vihan, K Gururaj, "Phytochemical analysis of some indigenous plants potent against ectoparasite" Asian J Exp Biol Sci. 2(3), (2011), 506-509
11. Sooad Al-Diahan , Manar Al-Faham, Nora Al-Shawi, Rawan Ainayaman, Amal Brrawi, Seema Zargar," Antibacterial activity and Phytochemical screening of some medicinal plants commonly used in Saudi Arabia against pathogenic micro-organisms" Journal of King Saud University Science. 25(2), 2013, 115-120.
12. Andri Cahyo Kumoro, Masitah Hasan, "Extraction of herbal components- the case for supercritical extraction" TEKNIK 29(3), 2008, 180-183.
13. M Sayyed, M Khan, N Devanna, Y H syed, J A Ansari, "pharmacognostical and Phytochemical investigation of the whole plant of *swertia chirata and Hemidesmus indicus"* Journal of pharmaceutical and biosciences, 4(2013) , 141-145.
14. Panchawas S, Sisodia S.S, "Evalution o anti-diarrhoeal activity of stem bark extracts of *ficus religiosa* prepared by different methods of extraction" International Journal of pharmaceutical abd biological archiss, 2012, 3 (1), 218-222.
15. Praveen Dahiya and Sharmishtha purkayastha, "phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistance bacteria from clinical isolates" Indian journal of pharmaceutical science, 74(5), 2012, 443-450.
16. K. Sahayaraj and C. Ravi, " Preliminary Phytochemistry of *Ipomea carnea Jacq* and *Vitex negundo Linn* leaves" , international journal of chemical sciences, 6 (1), 2008, 1-6.
17. P Bigoniya, C S Singh, B Srivastave, "Phmacognostical and physico-chamical standardization of *Syzygium Cumin and Azardirachta indica* seeds" Asian pacific journal of tropical biomedicine, (2012), S 290-S 295.