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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF TERMINALIA CHEBULA, MOMORDICA CHARANTIA, DREGEA VOLUBILIS PLANT EXTRACTS.

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Abstract

The preset study was framed out to evaluate the phytochemicals and the antibacterial activity of the fractions separated from crude extracts of the *Terminalia chebula, Momordica charantia, Dregea volubilis.* **Methods:** Phytochemical analysis was carried out using standard such as Mayer's test, Wagner's test, Dragendorff test, Hager's Test, Alkaline copper test, Lead acetate test, Ferric chloride (FeCl3) test to identify the presence of secondary metabolites such as alkaloids, Flavonoids, Tannins in the fractions separated from the above selected plants. **Results**: The test carried out for screening of phytochemicals was given positive. According to results we noticed the presence of different types of secondary metabolites such as Alkaloids, Phenols, Flavanoids, Tanins, Saponins, Carbohydrates, Amino acids in the leaf fractions. The fractions inhibited the growth of bacterial strains used in the study and exhibited antibacterial activity. The results were compared with standard reference drug ciprofloxacin at 10 mg/mL. Based on our results, it is noted that, the chloroform, ethanol and aqueous fractions are more active than n-hexane, toluene fractions. The least MIC value (12 and 16µg/ml) was recorded with ethanol and aqueous fractions of *Momordica charantia* respectively. **Conclusion:** fractions of separated from the *Dregea volubilis, Terminalia chebula, Momordica charantia* medicinal plants (leaves) had significant phtochemicals which are responsible for antimicrobial activity.

Introduction:-

India is bestowed with great diversity of medicinal plants which attribute to several pharmacological properties. According to World Health Organization, most of the drugs prescribed by the doctors are of plants. Owing to bio integrity and ease in isolation of bioactive compounds from the plants, the research in the drug discovery and development in the field of phyto chemistry have been gained an immense momentum. The extensive use of antibiotics towards bacterial and fungal infections produced multi drug resistant species which was threading people. Apart from the multidrug resistance, the side effect of the synthetic drugs is also one of the reason why the people

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are ascertaining for plant based medications which are of affordable and safe. Around 50,000 plants have been identified for its attributed medicinal properties. Plants offer a variety of natural compounds related to different molecular families which exhibits several Pharmacological activities such as anti-microbial, anti-oxidant, anti-cancer, anti-diabetic, anti- inflammatory etc., in humans.

Terminalia chebula commonly called as black- or chebulic myrobalan belonging to Combretaceae possess several pharmacological properties such as antidiabetic, antioxidant, anti microbial, anticancerous, antimutagenic, antiulcer, and wound healing activities (Kim et al., 2001; Suchalatha and Shyamala Devi, 2004; Rao and Nammi, 2006; Kannan et al., 2009; Vaibhav and ArunKumar, 2011; Prakash Chandra, 2012; Khan et al., 2015). In addition, Terminalia chebula is widely used in many Ayurvedic formulations for the treatment of infectious diseases like leucorrhoea, pyorrhea, chronic ulcer, and fungal infections of the skin.

Momordica charantia commonly called as bitter melon belonging to Cucurbitaceae family possess different types of pharmacological properties such as antimicrobial, anti-viral, anti-parasitic, anti-tumorous, anti-fertility, hypoglycemic and anti-carcinogenic. It also used in the treatment of several diseases like rheumatism, worms, gout, colic, illness of spleen and liver (Ng et al., 1992; Scartezzini and Speroni, 2000; Grover and Yadav, 2004; Duke, 1985; Beloin et al., 2005).

Dregea volubilis is a reference as a medicinal plant found in Ayurvedic, Siddha and Unani literature for the treatment of eye ailments including cataract Kumar and Totawar, 2015. ***The plant*** belongs to family Asclepiadaceae is extensively used in Indian traditional medicines. The leaf paste is used to treat cough, fever, severe cold and rheumatic pain Rajadurai, 2009. The preparations of leaf paste in combination with pepper and bark paste with hot milk is used to treat dyspepsia and primary urinary infections respectively (Pandikuma, 2007; Silija et al., 2008).

The preset study was framed out to evaluate the phytochemicals and the antibacterial activity of the fractions separated from crude extracts of the *Terminalia chebula, Momordica charantia, Dregea volubilis.*

Material And Methods:-

Plant collection and authentification:-

Plant Material Leaves of *Terminalia chebula*, *Momordica charantia*, *Dregea volubilis* Garuga pinnata were collected from Peddhapalli, Telangana. The species has been authenticated by Prof. V. Thirupathaiah taxonomist, Department of Biotechnology, Chaitanya Postgraduate College (Autonomous), Hanamkonda, Warangal Urban, Telangana, India.

Extraction Procedure:-

The collected plant material was chopped shade dried and grinded in homogenizer to coarse powder. The powder of each plant (100 grams) was used for extraction using soxhelt apparatus. The extraction was initially started with petroleum ether and terminated with methanol (low polar to high polar). The solvent was evaporated from crude extracts using distillation at 50^{o} C (Das et al., 2010; Nikhal et al., 2010).

Phytochemical analysis:-

Preparation of extracts:-

All the extracts were dissolved separately using dilute HCL acid and filtered. The filtered extract is used for detection of phytochemicals according to the methods described by Harbone 1998; Kokate1997.

Test for detecting alkaloids:-

Mayer's test:-

Mayer's reagent was freshly prepared by dissolving the mixture of mercuric chloride (1.36 g) and of potassium iodide (5 g) in 100 ml of water

Wagner's test:-

Dissolve 1 g of picric acid in 100 ml of H_2O. Wagner,s reagent and aqueous solution of iodine and potassium iodide.

Dragendorff test:-

This is how to make dragenorff's reagent.our (0.5g) of bismuth nitrate in a empty beaker.add (10ml) of concentration hydrochloric acid.pour (4g) of potassium iodide into another beaker ,add a little after and stir unit.

Hager's Test :-
Dissolve 2g of picric acid in 100ml of H_2O potassium antimonite. Boil 22g of potassium antimonite with 1liter of water until nearly all of the salt has dissolved, cool quickly and add 35ml of 10% potassium hydroxide.

Detection of Flavonoids:-
Alkaline copper test:-
Prepared mixing (0.5ml) copper sulphate,(0.5ml) sodium carbonate .Foline phenol reagent diluted 1:1 with distilled water. Sandard protein solution (100mg%)bovine serum albumin in (0.1N) NaOH.

Lead acetate test:-
Lead (11) acetate [pb (CH3COO) $_2$] also known as lead acetate, lead diacetate, plum bous acetate, sugar of lead, lead sugar salt saturin or Goulard's powder, is a white (crystalline chemical compound with a sweetish taste it is made by treating lead (2) oxide with acetic acid like other lead compounds, it is toxic. lead acetate is soluble in water and glycerin with water it forms the trohydrate, Pb [CH3COO] $_2$. 3H2O.

Detection of Tannins:-
Ferric chloride test use of ferric chloride (FeCl3) test for phenolics in general, alternatively, a portion at water extract is diluted with distilled H20 in ratio of 1:4 and few drops of 10% ferric chloride solution is added.

Antibacterial assay:-
Microbial strains:-
Gram positive Strains Methcillin- resistant Staphylococcus *aureus* (MRSA, NC TC 13616), Bacillus *subtilis* (ATCC 6633), Bacillus *cereus* , (ATCC 14579) and Gram negative strains Pseudomonas aeruginosa (ATCC 27853), Klebsiella pneumoniae (ATCC 43816), Escherichia coli (ATCC 8739), Proteus vulgaris (ATCC 13315), salmonella typhi (ATCC 19430) were procured from American Type Culture Collection, USA. Methcillin resistant Staphylococcus aureus was purchased from Culture Collections, UK. All bacterial strains stored at -80o C were streaked on Luria Bertani (LB) agar plates (Hi-media Laboratories, Mumbai, India) and incubated at 37^o C for 20 to 24 h. A few isolated colonies were selected from each plate and suspended in 5 ml of LB broth in sterile culture vessel. The vessel was plugged with cotton and incubated with gentle shaking (140 rpm) at 37^{o}C for 20 h.

Culture media:-
Nutrient Agar (NA) containing Bromocresol purple was used for the activation of Bacillus species while NA was used for the other antibacterial activity. Mueller Hinton Agar (MHA) was used for minimum inhibitory concentration (MIC).

Inoculation preparation:-
The bacterial strains were inoculated into sterilized nutritive broth and incubated at 35 ± 2°C for 24 h. The turbidity of the resulting suspensions are diluted with same nutritive broth to obtain a transmittance of 25% at 580 nm, this percentage was calculated spectrophotometrically using Bausch & Lomb spectrophotometer comparable to McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0 × 108 CFU/ml (a stock standard from which a working standard was drawn with concentration of 1 × 108 CFU/ml).

Agar well diffusion assay:-
The antibacterial activity of selected medicinal plant extracts was performed based on the guidelines of Clinical and Laboratory Standard Institute.

The selective medium was inoculated with the test organism and once the agar was solidified, the wells were created using a six millimeters diameter bork corer. The wells were filled with 25 µL of the plants extracts of 25, 50, 75 µg/ml concentrations. ciproflaxacin (10µg/ml) is used as positive control. The test was carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h.

Minimum inhibitory concentration (MIC):-
The MIC of the fractions was determined by diluting the varied concentrations (0.0-1000 µg/ml) of *Dregea volubilis, Terminalia chebula, Momordica charantia*. Equal volume of the fractions and nutrient broth were mixed in the test tube. Specifically 0.1ml of standardized inoculums of 1to 2 X 10^7 cfu/ml was added to each tube. The tubes were incubated aerobically at 37^o C for 18-24hrs. Two control tubes were maintained for each test batch. This

is as follows: tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculums (organism control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes.

Results:-

Phytochemical analysis:-

Table 1.0 represents the results of Phyto chemical analysis of *Dregea volubilis, Terminalia chebula, Momordica charantia* leaf fractions. The test carried out for screening of phytochemicals was given positive. According to results we noticed the presence of different types of secondary metabolites such as Alkaloids, Phenols, Flavanoids, Tanins, Saponins, Carbohydrates, Amino acids in the leaf fractions (Table 1.0).

Antibacterial activity:-

The fractions of *Dregea volubilis, Terminalia chebula, Momordica charantia* inhibited the growth of bacterial strains used in the study and exhibited antibacterial activity. The results were represented in the form of zone of inhibitions in table 2.0 and Minimum inhibitory concentration values in the table 3.0. The results were compared with standard reference drug ciprofloxacin at 10 mg/mL. Based on our results, it is noted that, the chloroform, ethanol and aqueous fractions are more active than n-hexane, toluene fractions. The n-hexane fraction showed least activity on tested bacterial strains. Among the Gram negative strains P. *vulgaris* was found least susceptible to all fractions, whereas, S. *typhi* was only susceptible to Dregea *volubilis* and Terminalia *chebula* ethanol fraction and showed resistance to other fractions. The ethanol and aqueous fraction of *Momordica charantia* exhibited highest zone of inhibition 28 and 26 mm against P. *aeruginosa* and E. *coli* respectively. Whereas, the ethanol and aqueous fractions of Terminalia *chebula* and *Dregea volubilis* were significant in the inhibition of all Gram positive strains (MRSA-Methicillin Resistant Staphylococcus *aureus*, B. *subtilis*, B. *cereus*) and two strains of Gram negative (P. *aeruginosa*, E. *coli*) (Table 2.0).

Minimum inhibitory concentration:-

Table 3.0 represents the results showed that the MIC values varied from 12~800µg/ml, for the tested fractions. The least MIC value (12 and 16µg/ml) was recorded with ethanol and aqueous fractions of *Momordica charantia* respectively. The MIC values of 12-16µg/ml with ethanol and aqueous fractions of *Momordica charantia* against P. *aeruginosa*, E. *coli* were found near to reference antibiotic on the corresponding microorganisms (Table 3.0).

Table 1:-Phytochemical analysis of fractions separated from Dregea volubilis, Terminalia chebula,

Species	Fractions	Alkaloids	Phenols	Flavanoids	Tanins	Saponins	Carbohydrates	Amino acids
Dregea volubilis	n- hexane	+	+	+	+	+	+	+
	Toluene	+	+	+	+	+	+	+
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	+	+
	Aqueous	+	+	+	+	+	+	+
Terminalia chebula	n- hexane	+	+	+	+	+	+	+
	Toluene	+	+	+	+	+	+	+
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	+	+
	Aqueous	+	+	+	+	+	+	+
Momordica charantia	n- hexane	+	+	+	+	+	+	+
	Toluene	+	+	+	+	+	+	+
	Chloroform	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	+	+
	Aqueous	+	+	+	+	+	+	+

indicates the presence of the compound

Table 2:-Antibacterial activity of fractions separated from Dregea volubilis, Terminalia chebula, Momordica

		Antibacterial activity in the form of Zone of inhibitions						
Species	**Fractions**	**MRSA**	**B. *subtilis***	**B. *cereus***	**P. *aeruginosa***	**E. *coli***	**P. *vulgaris***	**S. *typhi***
Dregea volubilis	n- hexane	12	08	09	18	--	--	--
	Toluene	15	10	14	11	16	10	--
	Chloroform	20	22	17	16	20	19	--
	Ethanol	24	18	20	22	24	22	15
	Aqueous	26	21	24	18	22	14	--
Terminalia chebula	n- hexane	--	08	10	10	--	--	--
	Toluene	20	14	17	15	19	15	--
	Chloroform	18	20	17	23	21	12	--
	Ethanol	22	26	23	20	25	18	12
	Aqueous	22	24	20	21	23	16	--
Momordica charantia	n- hexane	--	05	07	08	--	07	--
	Toluene	16	19	22	20	17	10	--
	Chloroform	21	17	19	18	25	14	-
	Ethanol	23	20	23	28	22	20	--
	Aqueous	20	23	21	25	26	18	--
Ciproflaxacin (10 mg/mL)		28	30	26	32	28	26	30

Table 3:- Minimum inhibitory concentrations of fractions separated from Dregea volubilis, Terminalia chebula, Momordica charantia Leaf extract

		Minimum inhibitory concentrations						
Species	**Fractions**	**MRSA**	**B. *subtilis***	**B. *cereus***	**P. *aeruginosa***	**E. *coli***	**P. *vulgaris***	**S. *typhi***
Dregea volubilis	n- hexane	>200	>500	>450	58.1	--	--	--
	Toluene	>100	>350	>150	>300	47.8	>350	--
	Chloroform	19.9	26.7	62.2	49.0	33.6	42.9	--
	Ethanol	20.4	55.6	30.8	25.1	21.8	29.1	>100
	Aqueous	12.9	20.4	19.0	55.8	26.8	>150	--
Terminalia chebula	n- hexane	--	>450	>350	>350	--	--	--
	Toluene	21.8	>150	68.1	>100	46.1	>100	--
	Chloroform	49.3	25.9	62.5	20.1	31.3	>200	--
	Ethanol	26.3	12.5	22.1	32.6	18.1	50.5	>200
	Aqueous	25.5	20.9	29.4	28.0	24.0	51.9	--
Momordica charantia	n- hexane	--	>800	>600	>450	--	>600	--
	Toluene	48.5	48.6	27.2	30.6	59.7	>350	--
	Chloroform	19.0	66.3	40.7	53.3	19.2	>150	-
	Ethanol	22.9	26.7	25.1	12.0	26.7	33.6	--
	Aqueous	27.0	23.6	18.5	18.6	16.0	52.6	--
Ciproflaxacin (10 mg/mL)		3.0	2.5	4.2	2.1	3.3	4.0	3.1

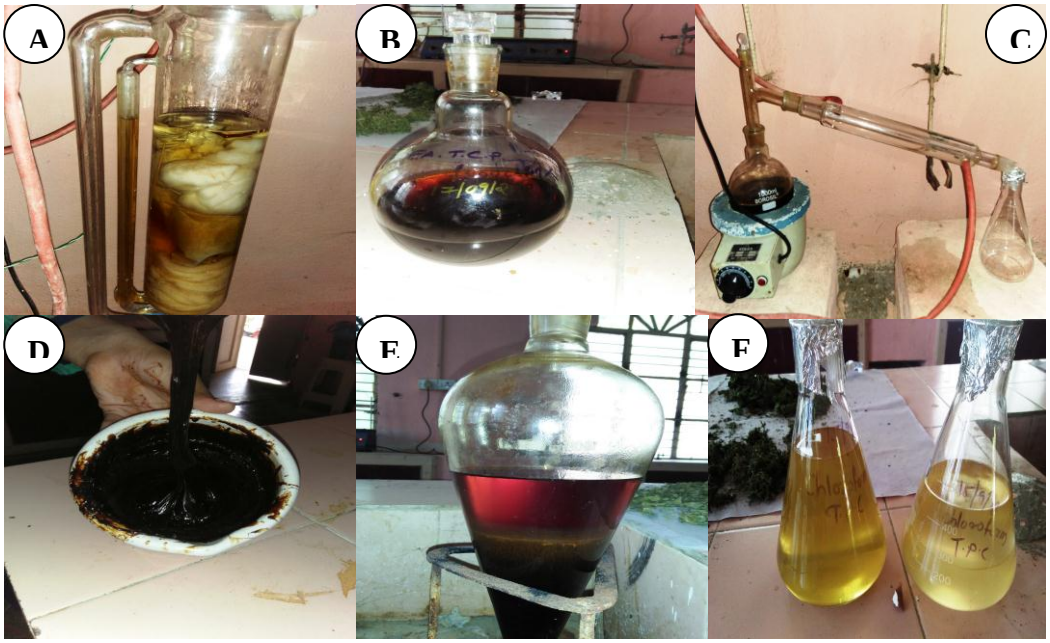

Fig 1:-A-Soxhlet apparatus packed with the plant material and processed for extraction using methanol, B-Solvent containing extract, C-Evaporation of solvent using distillation, D-Crude extract collected after distillation, E-Separation of fractions containing compounds based on polarity of compounds in separating funnel, F-Examples of fractions collected.

Discussion:-

The antibacterial activity of the fractions separated from crude extracts of *Dregea volubilis, Terminalia chebula, Momordica charantia* possessed significant inhibitory effects on all tested microorganisms. However, the sensitivity of the Gram positive and Gram negative strains is varied; perhaps this may be because of structural variation in the cell wall of bacteria. Based on the previous published reports, the high polar solvents which have good solubility of antimicrobial agents will easily penetrate through the lipopolysaccharide of bacterial outer membrane (primary permeability barrier of the cell) for the necessary action **(**Nikaido, 1996). Here we also agree with the above statement because the profound antimicrobial activity results especially on Gram negative organisms of the present work are obtained with only ethanol and aqueous solvents.

The secondary metabolites or phenolic compounds present in the fractions that are separated from the crude extracts of *Dregea volubilis, Terminalia chebula, Momordica charantia* may adopts several antibacterial mechanisms such as inhibition cell wall synthesis or causing energy depletion by accumulating in the cell wall or they increase the rate of permeability of cell membrane which leads to the loss of cellular constituents nor they disrupt membrane and change in the structure and function of cell organelles, consequently, resulting in the cell mutation and subsequent damage and death (Cowan, 1999; Marcucci et al., 2001; Conner, 1993; Kim et al., 1995).

Conclusion:-

According to the results obtained in the present work here conclude that the ethanol and aqueous fractions of separated from the *Dregea volubilis, Terminalia chebula, Momordica charantia*edicinal plants (leaves) had significant phytochemicals which are responsible for antimicrobial activity. However, there should be an extension of this work for isolation of principle compound that responsible for the activity.

Conflict Of Interest:-

Authors here declare no conflict of interest.

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