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INVITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF CALENDULA OFFICINALIS AGAINST MDR PATHOGENS

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ABSTRACT

The present study was carried out by evolution of *calendula officinalis* against MDR pathogens. In process to preparation of plant extract, organic solvent (methanol 80%) and aqueous solvent (hot water extraction) had been used. Calendula's leaves, roots, flower, branches, and stem had been used to study the antimicrobial activity. In calendula the best result had been observed in methanolic extract of roots with zone of inhibition of 39mm in case of *E.coli* as compare to other organic and aqueous extract of the sample. In case of MDR isolates the best result were seen when mixed extract had been used for AST (10µl of each methanolic extract) by agar well diffusion method with formation of ZOI of 20mm. The least concentration was found in methanolic extract of roots 0.01µg/ml. On Phytochemical screening of methanolic extract of calendula -tannin, cardiac glycoside and terpenoids were present in the form of secondary metabolites.

Key words: Phytochemical analysis, MIC, antimicrobial sensitivity test, secondary metabolites, calendula officinalis, MDR pathogens.

INTRODUCTION

Calendula officinalis is commonly known as marigold. It is an annual flower in Asteraceae family, having height up to 2 to 3 feet with presence of yellow and golden red flower and alternate branches [24]. The flower is native to Asia and southern Europe. According to the secondary sources calendula had been used medicinally since the 12th century, mainly in the Mediterranean. Calendula has been used topically to treat minor wounds, burns and other skin problems and naturopathy [33; 32], homeopathic [18], adverse effect of drugs [17] powder

form of the plant's petals is occasionally used as an inexpensive alternative to saffron for colouring and flavouring foods and consist essential oils [37].

In laboratory research, calendula found antidiabetic it has hypoglycaemic effects [1]. It have antihypersentitives [2], anti-inflammatory agents[3;4;5;6], Antiprotozal[7], Antispasmodic[8], it have antimicrobial activity [31]. Dietary Supplementation of *Calendula officinalis* counteracts the Oxidative Stress and Liver Damage Resulted from Aflatoxin [23]. In laboratory research work calendula has been shown to have activity against HIV [9], vesicular stomatitis virus and rhinovirus [10] as well as the Epstein - Barr virus [11]. Its other activity are immunosuppressant [12], sedative [13], have antioxidant [26], Antineoplastic [14; 15] cytotoxicity and genotoxicity [39; 22; 30]. *Calendula officinalis* produces a dual in *vitro* effect: cytotoxic anti-tumor activity and lymphocyte activation [38]. *Calendula* is toxic [34]. It has been found that in Phase III randomized trial of Calendula officinalis compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer [29], Management of skin toxicity during radiation therapy [19] and Treatment of varicose ulcer and skin lesions [28; 20] and exfoliative cheilitis [21] .

Calendula reported likely unsafe when used if pregnant and breast feeding, due to anecdotal reports of spermicidal and abortifacient effects [16]. Calendula has been reported high industrial value in food and Drug Company. The US Food and Drug administration FDA listed calendula on it's generally recognized as safe (GRAS) lists when used as a spice, natural seasoning, and flavouring it consist of fatty acids [35]. The present study is carried out to evaluate the antimicrobial activity of Calendula officinalis against various MDR pathogens.

MATERIALS AND METHODS

PREPARATION OF SAMPLE

Plant material

Here following parts of plant *calendula officinalis* is used that is flowers, roots, leaves, branches and stem. The plant was purchased and identified by the author from the local market of Gomati Nagar, Lucknow (U.P.) India.

Extraction of Bioactive Compounds from Sample

The plant (stem, roots, flower, branches, and leaves) was thoroughly washed. Every part of plant Sample was cut into pieces and were air-dried and ground into uniform powder using

grinder machine. The aqueous extract of each sample was prepared by heating 10gm of sample in 100 ml distilled water on water bath at 90°C for 2 hours. The organic extract was prepared by keeping the sample 5 gm of sample in dark bottle with 80% methanol for 48 hours. The extracts were filtered using whatman filter paper NO 42(125mm).After complete solvent evaporation, extract were dissolved in 100% dimethlysulphoxied (DMSO) (Merck (India) Ltd., Mumbai, India) to final concentration of 250mg/ml and stored at 4°C in labeled sterile eppendorf tubes for further use. The percentage of yield of plant extracts are shown in table no. 1 and 2.

Test Organism

The bacterial culture and fungal culture were collected from MRD life science lab Lucknow. They were subculture onto Petri plate containing nutrient agar media and potato dextrose agar media .The strain of bacteria selected to assess susceptibility pattern against extracts prepared in the study included are *Esherichiacoli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonellatyphi and Staphylococcus aureus.* Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tubes containing broth and incubated overnight at 37°C at shaker.

The fungal strain are *Aspergillusniger* and *Candidaalbicans* and these microorganism were also reactivated by transferring them into a separate test tube containing potato dextrose broth and incubated at 25°C for 48 hours.

The MDR culture isolates were isolated by serial dilution method of clinical waste soil sample.

Antimicrobial Assay

I.**Antibacterial assay**

The antibacterial activity of *calendula officinalis* extracts was determined by agar well diffusion method (Kirby Bauer method) against *Esherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus* and MDR culture obtained from clinical waste soil sample. 20ml of autoclaved Nutrient agar media was poured in sterile Petri plate. The pure isolates of each bacterium was sub cultured in Nutrient broth and incubated at 37°C at shaker for 24 hours. About 20µl of each test bacterium was inoculated by spread plate method in sterile agar plate so as achieve a confluent growth. Wells of 5mm diameter were punched into each Petri plate containing solidified agar media and filled with 50µl of plant extract and allowed to diffuse at room temperature for 2 hours.

The plates were then incubated in the upright position at 37°C for 24 hours. Well containing the same volume of distilled water serve as negative control while standard antibiotic (conc.10µg/ml of each antibiotic) Zoxan, Althrocin, Levoflox, Cephalexin serve as positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm. those inoculates which are resistant to more than 2 antibiotic were taken as MDR culture. The zone of inhibition formed by different bacteria and MDR isolates against antibiotic and different extracts are listed in table no 3, 4 and 5 respectively.

II. **Antifungal assay**

The antifungal activity of *calendula officinalis* extracts was also determined by agar well diffusion method (Kirby Bauer method) against *Aspergillus niger, Candidaal bicans.* 20ml of autoclaved Potato dextrose agar was poured in sterile Petri plate. The pure isolates of each fungus was sub cultured in Potato Dextrose broth and incubated at 25°C at shaker for 48 hours. About 20µl of each test fungus was inoculated by spread plate method in sterile agar plate so as achieve a confluent growth. Wells of 5mm diameter were punched into each Petri plate containing solidified agar media and filled with 50µl of plant extract and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 25°C for 48 hours. Well containing the same volume of distilled water serve as negative control. After incubation, the diameters of the growth inhibition zones were measured in mm. The zone of inhibition formed by different fungi against each extract are listed in table no 6.

DETERMINATION OF M.I.C. BY BROTH DILUTION METHOD

Based on the preliminary screening of methanol and aqueous extracts that revealed potent antimicrobial activity were further tested to determine the minimum inhibitory concentration (MIC) for bacterial and MDR isolates against each extract (table no. 7.1 to 7.5). The MIC of these extract were determine by broth dilution technique where 0.5ml (conc. 250mg/ml) of the extract were suspended in broth 3ml/ test tube and its serial dilution was done up to 6 test tubes. Two sets were prepared one working and the other was blank. The dilution factor was 1/6. The working set was inoculated with 20µl of test culture in each test tube an incubated at 37°C for 24 hours at shaker. After incubation the growth of bacterial isolates in the test tubes were observed as turbidity using spectrophotometer at 620 nm. The least concentration where no turbidity was observed was determined and noted as the MIC value.

PHYTOCHEMICAL ANALYSIS OF THE EXTRACT

Chemical test for the screening and identification of bioactive chemical constituents in the *calendula officinalis* were carried out in methanolic extract of sample using the standard methods of sofowara (1993), Trease and Evans (1989) and Harbone (1973).

Tannins

0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test tube and filtered 0.1% $FeCl_3$is added to the filtered samples and observed for brownish green or a blue black coloration which shows the presence of tannins.

Anthraquinones: About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH were added tothe mixture and heat. Formation of rose-pink color indicates the presence of anthraquinones.

Borntreger's test was also used for the detection of anthraquinones. 5 g of plant extract was shaken with 10 ml of Benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet color in the ammonical (lower) phase indicated the presence of free hydroxyl anthraquinones.

Glycosides: 0.5 g of solvent extract was dissolved in 2.0 ml of glacial acetic acid containing one drop of $FeCl_3$ Solution. This was then under laid with 1.0 ml of concentrated H_2So_4. A brown ring obtained at the interface indicated the presence of glycosides.

Test for cardiac glycosides (Keller-Killani test): 5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Reducing sugar- the extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling solution A and B for minutes. An orange red color precipitate indicates the presence of reducing sugar.

Saponins: Froth test About 2.5 g of the plant material was extracted with boiling water. After cooling, the extract was shaken vigorously to froth and was then allowed to stand for

15-20 min and classified for saponin content as follows: no froth = negative; froth less than 1 cm = weakly positive; froth 1.2 cm high = positive; and froth greater than 2 cm high = strongly positive.

Flavonoids: Shinoda test The solvent extract (5 ml, corresponding to 1 g of plant material) was treated with a few drops of concentrated HCl and magnesium turnings (0.5 g). The presence of flavonoids was indicative if pink or magenta – red color developed within 3 min.

Phlobatanins: The extract (0.5 g) was dissolved in distilled water of the extract and filtered. The filtrate was boiled with 2% HCl Red precipitate shows the presence Phlobatanins.

Terpenoids: Salkowski test: Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Phenols: Ferric chloride test The Solvent plant extract was treated with few drops of neutral ferric chloride solution, formation of bluish.

RESULT

I.Plant material

The dried powder of following parts of calendula officinalis was obtained Leaves, branches, roots, stems, and flower.

II.Preparation of the extract

Extraction was carried out using methanol 80%, and hot water extraction.

- **Table no.1 for Methanolic extraction**

Sample / extract	Yield obtained (gm/ 5gm of sample)
Leaves	1.639gm
Branches	.771gm
Roots	.992gm
Flowers	1.223gm
Stems	1.023gm

- **Table no.2 for Hot water extraction**

Extract	Yield obtained (gm/10gm of sample)
Leaves	1.825gm
Flowers	.227gm

III.Test organisms

Quadrant plates and broths are prepared for the cultures of *Pseudomonas, E.coli, B. subtilis, S. aureus, and S. typhi.*

Figure 1 quadrant culture of B.subtilis.

IV. Antibiogram of calendula officinalis

- **Antibacterial activity**: the antibacterial activity of all the plant extract of calendula officinalis were assayed against *Pseudomonas aeruginosa, Escherichia coli, staphylococcus aureus, B.subtilis, and S.typhi. By agar well diffusion method.*

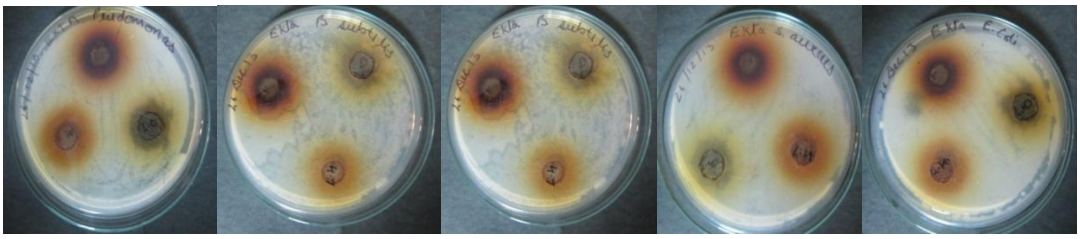


Figure 2 AST of Pseudomonas E.coli, S.aureus, B.subtilis and S.typhi against methanolic extract of leave and flower and leave hot water extract.

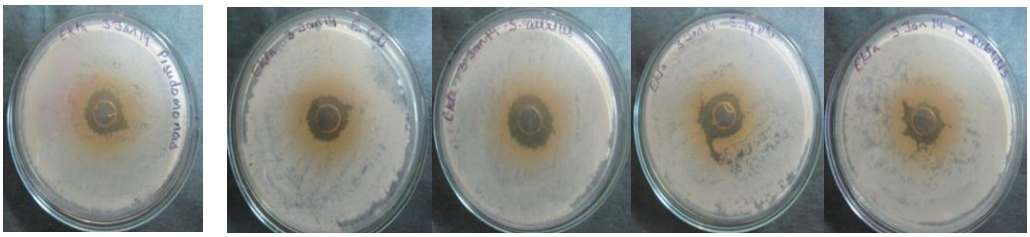


Figure 3 AST for Pseudomonas, E.coli, S.aureus, B.subtilis and S.typhi against methanolic extract of roots of calendula.

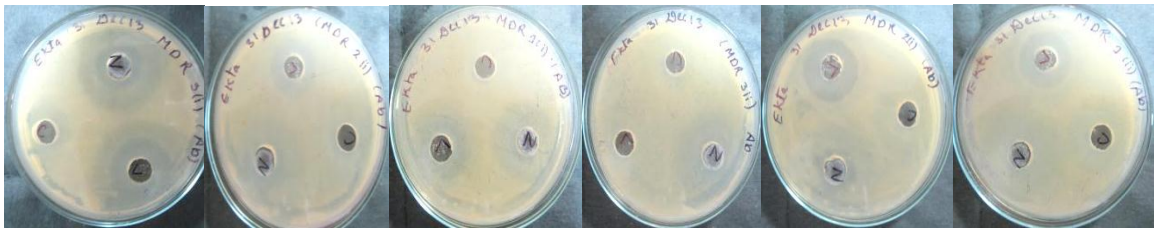


Figure 4 AST for sample taken from hospital waste to check is it MDR culture or not, as it is showing resistance to presence of more than 2 drugs it can be said that it is MDR culture. Drug used are zoxan (Z), Cephalexin(C), and levoflox (L).

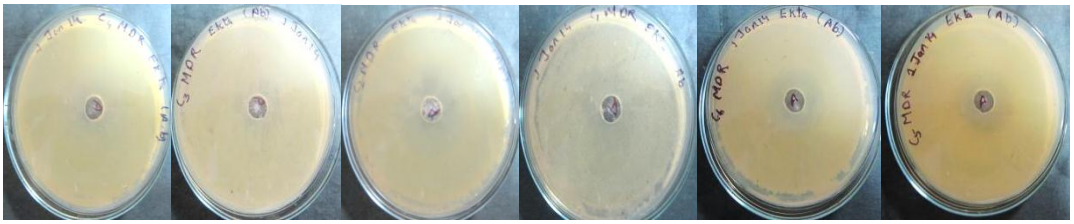


Figure 5 AST for the sample collected from hospital to check is it MDR pathogen or not. Its sensitivity is checked against drug Althrocin; it is partially sensitive to its presence.

From the study of above result it is conclude that the bacterial colonies isolated from the hospital waste are resistant to the presence of antibiotic as it is partially sensitive to its presence.

Figure 6 AST for MDR isolates against methanolic extract of flower and leaves and hot water extract of leaves, from the zones of inhibition form in the plate it can conclude that MDR pathogen are sensitive to the presence of extract.

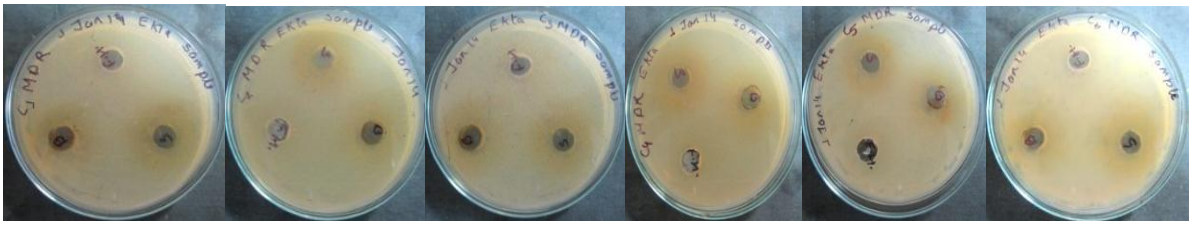


Figure 7 AST for MDR isolates against methanolic extract of stem (S), branches (B) andhot water extract of flower.

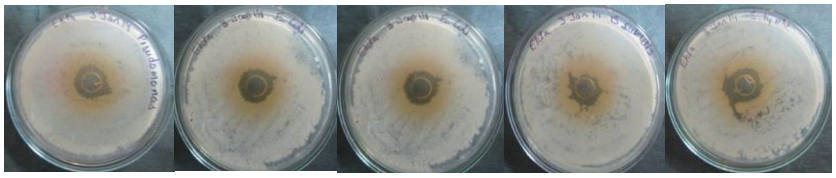


Figure 8 AST for S.aureus, Pseudomonas, E.coli, S.typhi, and B.subtilis against methanolic extract of roots.

From the above figure it is concluded that all the bacteria *S.aureus, Pseudomonas, E.coli, S.typhi and B.subtilis* are highly sensitive to the methanolic extract of roots.

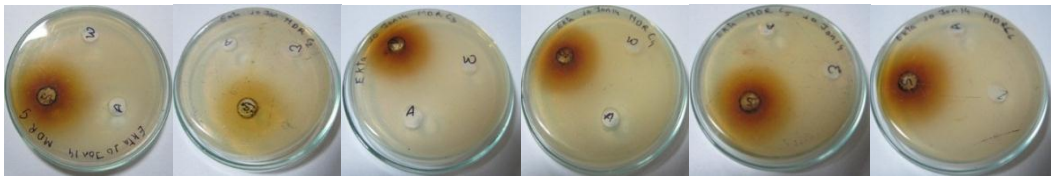


Figure 9 AST for MDR isolates against negative control water (W), positive control antibiotic levoflox (A) and extract of all sample(S).

Figure 10 AST for negative control water (W), antibiotic levoflox (A) and mixed extract of sample (s), against pathogen Pseudomonas, E.coli, S.aureus, B. subtilis, S. typhi.

Antifungal assay

The antifungal activity of extract of the entire sample (flowers, roots, stems, branches and leaves) were assayed against *Aspergillus niger* and *Candida albicans* by agar well diffusion method.

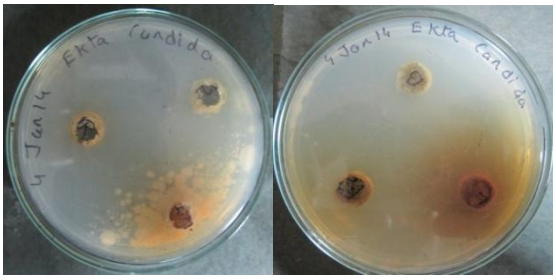


Figure 11 AST for Candida against methanolic extract of roots, stems, branches, leaves flower and hot water leave extract. Candida showing sensitivity against methanolic extract of stem, root, leave and flower.

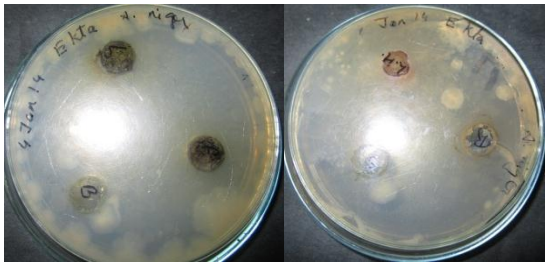
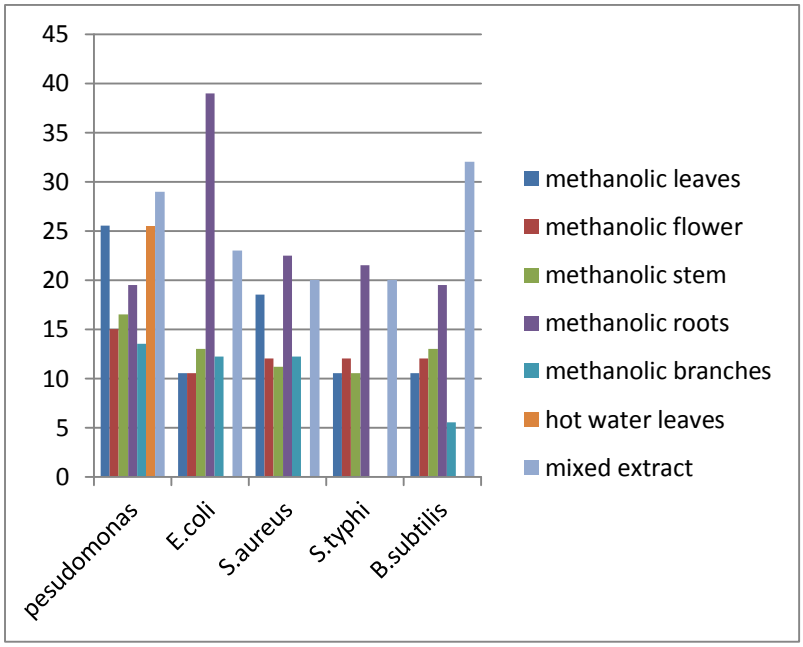


Figure 12AST for A.niger against methanolic extract of roots, stems, branches, leaves flower and hot water leave extract.

TABLES AND GRAPH

Table no.3 zone of inhibition form in the plates against methanolic extract of stem (SM), roots (RM), flower (FM) leaves (LM) branches (BM) and hot water extract of leaves (LW) for pseudomonas, S.aureus, E.coli, S.typhi and B. subtilis and mixed extracts.

Pathogen	Zone of inhibition in mm						
	Fm	Mixed extract	Lm	Bm	Sm	Rm	Lw
Pseudomonas	15	29	25.5	13.5	16.5	19.5	25.5
E.coli	10.5	23	10.5	12.5	13	39	00
S.aureus	12	20	18.5	12.2	11.2	22.5	00
B.subtilis	12	20	10.5	5.5	13	19.5	00
S.typhi	12	32	10.5	00	10.5	21.5	00



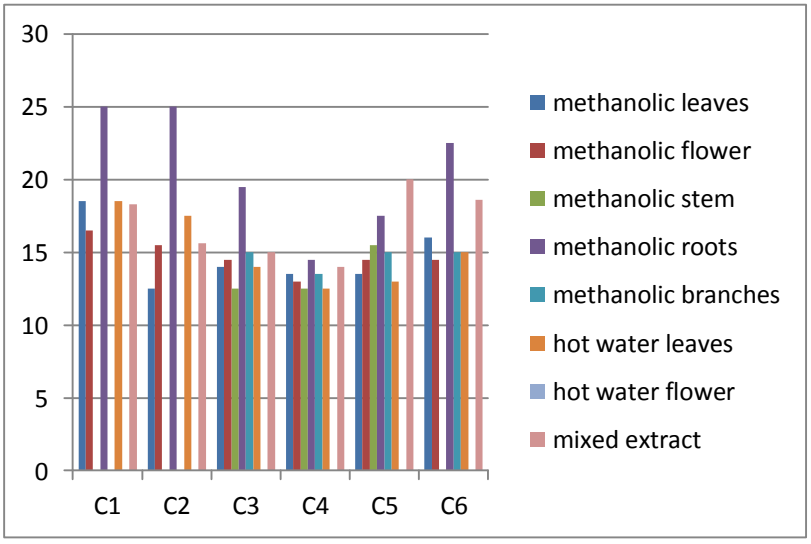
Graph for table no. 4

Table no. 4 zone of inhibition form in plates against antibiotics to isolate MDR culture.

culture	Activity of antibiotic (R) resistance (S) sensitive			
	zoxan	Cephalexin	Levoflox	Althrocin
C_1	R	R	R	R
C_2	R	R	S	R
C_3	R	R	S	R
C_4	R	R	R	R
C_5	R	R	S	R
C_6	R	R	R	R

Table no 5 zone of inhibition form in plates against extract of calendula for MDR isolates. Methanolic extract of stem (SM), roots (RM), flower (FM) leaves (LM) branches (BM) and hot water extract of leaves (LW) and flower (FW and mixed extract)

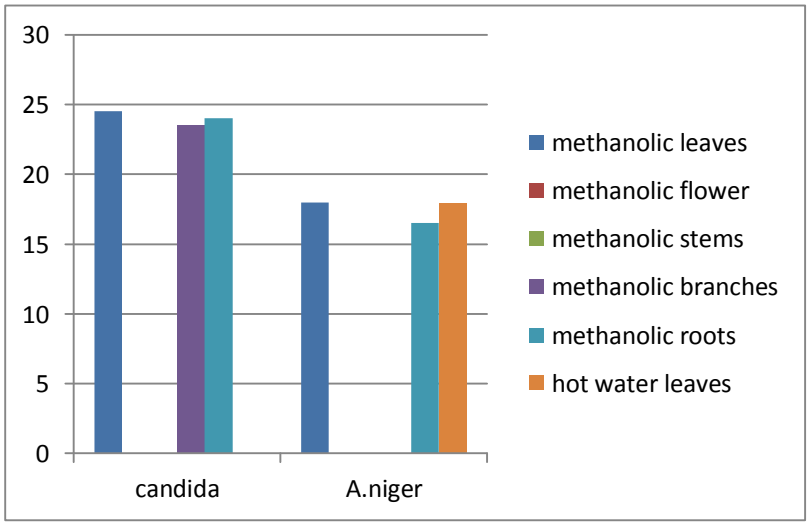
Culture	Zone of inhibition form in mm							
	LM	FM	SM	BM	RM	LW	FW	Mixed extract
C_1	18.5	16.5	R	R	25	18.5	00	18.3
C_2	12.5	15.5	R	R	25	17.5	00	15.6
C_3	14	14.5	12.5	15	19.5	14	00	15
C_4	13.5	13	12.5	13.5	14.5	12.5	00	14
C_5	13.5	14.5	15	15	17.5	13	00	20
C_6	16.5	14.5	R	15	22.5	15	00	18.6



Graph for table no 6 zone of inhibition at y axis and culture at axis x.

Table no 7 for zone of inhibition of fungi against methanolic extract of stem (SM), roots (RM), flower (FM) leaves (LM) branches (BM) and hot water extract of leaves (LW) and flower (FW)

Culture	Zone of inhibition in mm					
	LM	FM	SM	RM	BM	LW
A.niger	18	0	0	16.5	0	18
Candida	24.5	0	0	24	23.5	0



Graph for table no. 7

Table 7.1of O.D. for MIC of methanolic leaves extract

Culture	O.D. at 620nm	Concentration in mg/ml
C_1	0.01	125
C_2	0.06	2.83
C_3	0.01	0.471
C_4	0.36	0.691
C_5	0.35	0.115
C_6	0.40	0.01

The least concentration for leaves observed is 0.471mg/ml at O.D. 0.01.

Table no. 7.2 O.D. for MIC of methanolic flower extract

Culture	O.D. at 620nm	Concentration in µg/ml
C_1	**0.0**	**96.375**
C_2	0	16.06
C_3	0.08	2.67
C_4	0.37	0.44
C_5	0.43	0.075
C_6	0.39	0.0123

The MIC for flower is 0.0123 µg/ml which is observed at O.D. 0.39.

Table no 7.3 O.D. of MIC of methanolic branches at O.D. 620nm.

Culture	O.D. at 620nm	Concentration in µg/ml
C_1	00	1240
C_2	0.04	206.6
C_3	0.15	34.3
C_4	0.31	5.71
C_5	0.38	0.95
C_6	0.27	0.12

The MIC for branches is 0.12 µg/ml which is observed at O.D. 0.27nm.

Table no 7.4 O.D. of MIC of methanolic stems at O.D. 620

Culture	O.D. at 620nm	Concentration in µg/ml
C_1	00	1528.85
C_2	0.05	254.8
C_3	0.22	42.46
C_4	0.22	7.07
C_5	0.22	1.17
C_6	0.20	0.19

The MIC for stems is 0.19µg/ml which is observed at O.D. 0.20nm.

Table no. 7.5 O.D. of MIC of methanolic roots at O.D. 620

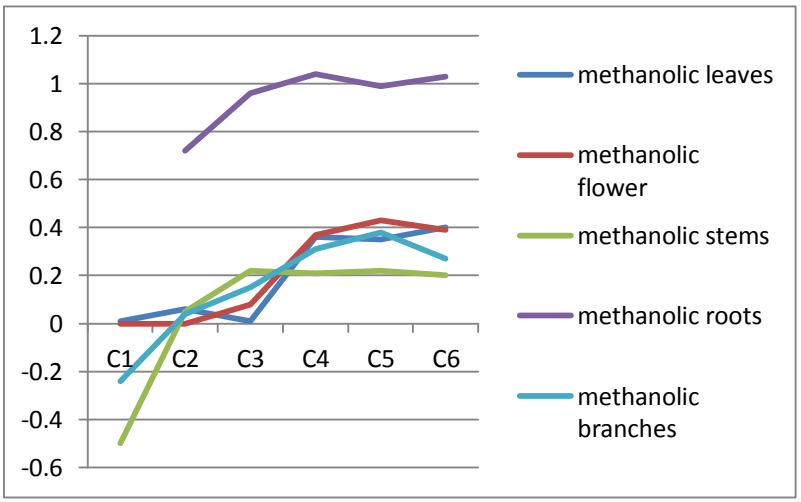
Culture	**O.D. at 620nm**	**Concentration in µg/ml**
C_1	0.06	127.87
C_2	0.72	21.3
C_3	0.96	3.55
C_4	1.04	0.592
C_5	0.99	0.0986
C_6	1.03	0.016

The MIC for methanolic roots is 0.09µg/ml at O.D. 0.99nm.

Table no. 9 phytochemical results

Chemical component	**Methanolic extract**
Tannins	+
Anthraquinones	-
Cardiac glycoside	+
Reducing sugar	-
Saponins	-
Flavonoids	-
Phlobatanins	-
Terpenoids	+
Phenols	-

Phytochemical screening of methanolic extract of roots stems, branches, leaves and flower.



Graph for table no 7.1 to 7.5 of MIC against methanolic extract.

DISCUSSION

Calendula officinalis is well known herbs used ayuverdic traditional medicine for their effectiveness against wide range of diseases including skin infections due to the advantage of the diversity of secondary metabolites responsible for their antibacterial activity Calendula officinalis seeds influences seedling vigor and essential oil yield in flowers [27]. The different levels of salt stress affect the germination of Calendula officinalis [23]. Moreover, the number of immune compromised patients is on the increase due to great progress in the field of intensive care, organ transplantation and AIDS[9]. HIV-positive patients have developed resistance to treatment with existing antibiotics. Despite the existence of potent antibacterial agents, the appearance of resistant or multi-resistant strains imposes the need for a permanent search and development of new drugs. Calendula has been widely used as tincture for healing wounds and treating burns in homeopathy extensively [18]. The aqueous decoctions are reported to be antibacterial agents in traditional system of medicine.

The results of the present work investigation reveal the antimicrobial nature of methanolic and aqueous extract of *calendula officinalis.* The leaves, flower, stems, branches and roots of *calendula officinalis* was extracted in dried powder form using aqueous solvent (water) and organic solvent (methanol 80%). The maximum extraction is found in 80% methanol extract of leaves which is 1.639gm followed by 1.223gm of stems, 1.023 gm of roots, branches .992gm and the least extraction is observed in methanolic extract of flower that is 0.771gm in per 5gm of the sample.

The antimicrobial activity was assayed by agar well diffusion method (Kirby Bauer method) against all five pathogens *E.coli, B.subtilis, Pseudomonas aeruginosa, S.typhi and S. aureus* for aqueous and organic solvent of *Calendula officinalis.* After analyzing different zone of inhibition of different solvents, the pattern of microbial sensitivity was found maximum in *E.coli* with 39mm ZOI as in case of root methanolic extract and *Pseudomonas* with 25.5mm zone of inhibition as in case of leave organic and aqueous extract. Methanolic extract of flower is also showing good antimicrobial activity with zone of inhibition (in mm) of 15, 10.5,12,12,12 for *Pseudomonas, E.coli, S.aureus, B.subtilis,* and *S.typhi* respectively.
The least antimicrobial activity is observed in aqueous extract of leaves and flower with no zone of inhibition in *E.coli, S.aureus, B.subtilis,* and *S.typhi,* But showing good zone of inhibition in case of *Pseudomonas* 25.5mm. And no antimicrobial sensitive is found in *E.coli, B.subtilis, and S.typhi* with no zone of inhibition as in case of methanolic extract of branches.

To isolate MDR culture from the sample obtained from Rammanohar Lohia hospital, spreading and then streaking is done in order to get pure isolated colonies. Broth is prepared for the pure isolates and to confirm that is it MDR or not. Antimicrobial sensitivity test is done by agar well diffusion method. The culture named C1, C2, C3, C4, C5, C6 found resistant to 4, 3, 3, 4, 3, 4 drugs respectively. On this basis it can be said that they are MDR culture. The drugs used are Althrocin, Levoflox, Zoxan and Cephalexin out of this in case of levoflox best sensitivity is seen among all the MDR isolates. In case of MDR isolates the maximum ZOI is form in C1, C2 and C6 that is 25, 25 And 22.5 respectively as in case of mixed extract (10µl of each methanolic extract is used together in the AST) . C1, C2 and C6 are resistant to presence of methanolic extract of stem and branches. Antimicrobial activity is absent in case of hot water extract of flower. Methanolic extract of roots and leaves showing good antimicrobial activity in case of MDR isolates.

The least concentration was found for methanolic root extract .01µg/ml against C6 isolate of MDR culture by broth dilution method for MIC. Least concentration for methanolic stem, branches, flower and leaves were 0.19 µg/ml, 0.120 µg/ml, 0.0123µg/ml, and 0.471mg/ml respectively.

On screening for phytochemical analysis of the methanolic extract of the calendula it is found that secondary metabolites tannin, cardiac glycoside, and Terpenoids were present, spectrophotometric analysis of calendula also result in presence of secondary metabolites[25].

Herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs [36]. Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts obtained using same herb with different solvents will be different.

CONCLUSION

In this whole data of project, it was concluded that the maximum yield was obtained in organic solvent extraction in comparison to aqueous solvent extraction. The best results of antimicrobial screening were found in *E.coli and S.typhi* methanolic extract of roots with zone of inhibition of 39mm and 21.5mm respectively.

In isolates of MDR culture the best activity is seen in mixed methanolic extract of all sample (10µl of each) with maximum zone of inhibition of 20 mm as in case of C5 which is resistant to all 4 drug used (Althrocin, Levoflox, Zoxan, and Cephalexin) .

The least concentration observed is .01µg/ml as in case of methanolic extract of roots in MIC by broth dilution method.

On phytochemical screening of methanolic extract, secondary metabolites tannin, cardiac glycoside, and Terpenoids were found present.

REFERENCES

1. Ahmad.I and Beg.A.J.antimicrobial and phytochemical studies on 45 indian medicinal plants against multidrug resistance human pathogens. J. Ethnapharma.vol.2001.
2. Akhtar, N., Zaman, S. U., Khan, B. A., Amir, M. N., and Ebrahimzadeh, M. A. Calendula extract: effects on mechanical parameters of human skin. ActaPol.Pharm. 2011; 68(5):693-701.
3. Amitpandey, afsheen2, firdousara, sudeepkumartiwari,isolation and characterization of multi drug resistance cultures from waste water,journal of pharmaceutical and biomedical sciences, 2011; 12 (14).
4. Amitpandey, firdousara, afsheensiddiqui, sudeepkumartiwari, optimization of culture conditions of mdr pathogens isolated from waste water, journal of pharmacy research 2012;5(3):1795-1798

5. Andersen, F. A., Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., Liebler, D. C., Marks, J. G., Jr., Shank, R. C., Slaga, T. J., and Snyder, P. W. Final report of the Cosmetic Ingredient Review Expert Panel amended safety assessment of Calendula officinalis-derived cosmetic ingredients. Int.J.Toxicol. 2010; 29(6 Suppl):221S-2243.
6. Anonymous. Final report on the safety assessment of Calendula officinalis extract and Calendula officinalis. Int J Toxicol 2001; 20(2):13-20.
7. AvinashMarwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur,Evidence of the association of begomovirus and its betasatellite with the yellow vein disease of an ornamental plant Calendula officinalis (pot marigold) in Rajasthan, India: Molecular, Sequence and Recombination analysis, Journal of Advances in Biology Vol. 1, No. 1.
8. Bauer.AW, Kirby. WM, Sheries JC, TurckM.Antibiotic. Susceptibility testing by a Standardized single disc method. Am Jclin Pathol.1966; 45:49-58.
9. BP Muley, SS Khadabadi and NB Banarase Phytochemical Constituents and Pharmacological Activities of Calendula officinalisLinn (Asteraceae): A Review Tropical Journal of Pharmaceutical Research, October, 2009; 8 (5): 455-465.
10. Chargari, C., Fromantin, I., and Kirova, Y. M. [Importance of local skin treatments during radiotherapy for prevention and treatment of radio-induced epithelitis]. Cancer Radiother. 2009; 13(4):259-266.
11. Cravotto, G., Boffa, L., Genzini, L., and Garella, D. Phytotherapeutics: an evaluation of the potential of 1000 plants. J.Clin.Pharm.Ther. 2010; 35(1):11-48.
12. de, Andrade M., Clapis, M. J., do Nascimento, T. G., Gozzo, Tde O., and de Almeida, A. M. Prevention of skin reactions due to teletherapy in women with breast cancer: a comprehensive review. Rev.Lat.Am.Enfermagem. 2012; 20(3):604-611.
13. Duran, V., Matic, M., Jovanovc, M., Mimica, N., Gajinov, Z., Poljacki, M., and Boza, P. Results of the clinical examination of an ointment with marigold (Calendula officinalis) extract in the treatment of venous leg ulcers. Int.J.Tissue React. 2005; 27(3):101-106.
14. Eva Jiménez-Medina, Angel Garcia-Lora, Laura Paco1, Ignacio Algarra,Antonia Collado3 and Federico Garrido, A new extract of the plant calendula officinalisproduces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation BMC Cancer 2006, 6:119 doi:10.1186/1471-2407-6-119.
15. Gol'dman, I. I. [Anaphylactic shock after gargling with an infusion of Calendula]. Klin.Med.(Mosk) 1974;52(4):142-143.
16. Harborne JB., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd edition). Chapman and Hall Co., New York, pp.1-302 (1998).

17. Jeschke, E., Ostermann, T., Luke, C., Tabali, M., Kroz, M., Bockelbrink, A., Witt, C. M., Willich, S. N., and Matthes, H. Remedies containing Asteraceae extracts: a prospective observational study of prescribing patterns and adverse drug reactions in German primary care. Drug Saf, 2009; 32(8):691-706.
18. Kassab, S., Cummings, M., Berkovitz, S., van, Haselen R., and Fisher, P. Homeopathic medicines for adverse effects of cancer treatments. Cochrane.Database.Syst.Rev. 2009; (2):CD004845.
19. Kumar, S., Juresic, E., Barton, M., and Shafiq, J. Management of skin toxicity during radiation therapy: a review of the evidence. J.Med.ImagingRadiat.Oncol. 2010;54(3):264-279.
20. Kundakovic, T., Milenkovic, M., Zlatkovic, S., Nikolic, V., Nikolic, G., and Binic, I. Treatment of venous ulcers with the herbal-based ointment Herbadermal(R): a prospective non-randomized pilot study. Forsch.Komplementmed. 2012; 19(1):26-30.
21. Lúcia Helena DenardiRoveroni-Favaretto, Karina Bortolin Lodi and Janete Dias Almeida Topical Calendula officinalis L. successfully treated exfoliative cheilitis: a case report Cases Journal 2009, **2**:9077 doi:10.1186/1757-1626-2-9077.
22. M mithirai and r arulvel, extraction and cytotoxicity Potential of marigold flower (calendula officinalis) against breast cancer cell line (mcf 7) int. J. Lifesc. Bt & pharm. Res. 2013; 2(3) : 2013.
23. Mohamed A. Hamzawy, Ezzeldein S. M. El-Denshary, Nabila S. Hassan, Fathia A. Mannaa, andMosaad A. Abdel-Wahhab, Dietary Supplementation of Calendula officinalis Counteracts the Oxidative Stress and Liver Damage Resulted from Aflatoxin,Hindawi Publishing Corporation ISRN Nutrition Volume 2013, Article ID 538427, 9 pages.
24. Mohammad HoseinGharineh, Hamid Reza Khoddami, Mahmoud Rafieian-kopaei, The influence of different levels of salt stress on germination of marigold (Calendula officinalis L.), International Journal of Agriculture and Crop Sciences, IJACS/2013/5-14/1581-1584.
25. Monica Butnariu and Cristina ZepaCoradini Evaluation of Biologically Active Compounds from Calendula officinalis Flowers using Spectrophotometry, Butnariu and Coradini Chemistry Central Journal 2012, 6:35.
26. NedaBabaee, DariushMoslemi, Mohammad Khalilpour, FatemehVejdani, YasamanMoghadamnia, Ali Bijani, Mahmoud Baradaran, Mohammad TaghiKazemi, AsiehKhalilpour, Mahdi Pouramir and Ali Akbar Moghadamnia, Antioxidant capacity of

calendula officinalis flowers extract and prevention of radiation induced oropharyngealmucositis in patients with head and neck cancers: a randomized controlled clinical study, DARU Journal of Pharmaceutical Sciences 2013, 21:18.

27. NegarValizadeh, BahramMirshekari, NesetArslan, Enrichment of marigold (Calendula officinalis) seeds influences seedling vigor and essential oil yield in flowers,International Journal of Biosciences,Vol. 3, No. 12, p. 175-179, 2013
28. Neto, J. J., Fracasso, J. F., Neves, M. D. C. L. C., and et al. Treatment of varicose ulcer and skin lesions with calendula. Revista de Ciencias Farm Sao Paulo 1996;17:181-186.
29. Pommier, P., Gomez, F., Sunyach, M. P., D'Hombres, A., Carrie, C., and Montbarbon, X. Phase III randomized trial of Calendula officinalis compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. J Clin.Oncol. 4-15-2004;22(8):1447-1453.
30. Ramos, A., Edreira, A., Vizoso, A., Betancourt, J., Lopez, M., and Decalo, M. Genotoxicity of an extract of Calendula officinalis L. J Ethnopharmacol. 1998;61(1):49-55.
31. Roopashree TS, Raman Dang , Shobha Rani RH, Narendra C, Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordicacharantia and Calendula officinalis International Journal of Applied Research in Natural Products Vol. 1(3), pp. 20-28, Sep/Oct 2008.
32. Sarrell EM, Mandelberg A, and Cohen HA. Efficacy of naturopathic extracts in the management of ear pain associated with acute otitis media. Arch Pediatr Adolesc Med 2001;155(7):796-799.
33. Sarrell, E. M., Cohen, H. A., and Kahan, E. Naturopathic treatment for ear pain in children. Pediatrics 2003;111(5 Pt 1):e574-e579.
34. Silva EJ, Gonçalves ES, Aguiar F, Evêncio LB, Lyra MM, Coelho MC, FragaMdo C, Wanderley AG, Toxicological studies on hydroalcohol extract of Calendula officinalis L.Phytother Res. 2007 Apr;21(4):332-6
35. Francisc V Dulf, Doru Pamfil, Adriana D Baciu and Adela Pintea, Fatty acid composition of lipids in pot marigold(Calendula officinalis L.) seed genotypes Dulf et al. Chemistry Central Journal 2013, 7:8
36. Trease, G.E. and W.C. Evans, A textbook of pharmacognosy. 14th Ed. BaillièreTindall Ltd. London 1996.
37. Zilda Cristina Gazim, Claudia MoraesRezende, Sandra Regina Fraga, Benedito Prado DiasFilho, CelsoVataru Nakamura, Diógenes Aparicio Garcia Cortez, Analysis of the

essential oils from Calendula officinalis growing in Brazil using three different extraction procedures Brazilian Journal of Pharmaceutical Sciences vol. 44, n. 3, jul./set., 2008.

38. Eva Jiménez-Medina, Angel Garcia-Lora, Laura Paco, Ignacio Algarra, Antonia Collado and Federico Garrido, A new extract of the plant calendula officinalis produces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation, BMC Cancer 2006, 6:119 doi:10.1186/1471-2407-6-119, 2006
39. M Mithirai and R arulvel, extraction and cytotoxicity potential of marigold flower (calendula officinalis) against breast cancer cell line (MCF 7), Int. J. LifeSc. Bt & Pharm. Res., Vol. 2, No. 3, July 2013