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EFFECT OF CALOTROPIS PROCERA INHIBITS HUMAN CANCER CELL LINES, PATHOGENIC BACTERIA AND FUNGI IN- VITRO

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ABSTRACT

Objective This research study was carried out to evaluate the qualitative and quantitative effect of *Calotropis procera* roots ethanol extract on pathogenic bacterial and fungal strains along with cancer cell lines.

Methods Extraction was done by Soxhlet method of *Calotropis procera* roots in ethanol. Phytochemical estimation of selected seven secondary metabolites i.e. alkaloids, flavonoids, anthroquinone, saponins, terpenoids, tannins and cardiac glycosides was done followed by extraction as per the protocols of Kokate [1]. Microbiological study of *Calotropis roots* ethanol (CRE) was done by dilution method and gradient plate method on five pathogenic bacteria and five fungal strains [13]. Total activity calculated by using MIC. Inhibitory potential was checked by SRB (Sulphorhodamine B) assay on six kinds of cancer cell lines i.e. Breast (MCF7), Breast metastatic (MDMAMB468), Lung (A549), Lung metastatic (NCIH358), colon (DLD1), colon metastatic (SW620) [2].

Results *Calotropis* roots ethanol extract is a non-polar solvent extract and considered as the best solvent to extract the maximum number of secondary metabolites like alkaloids, saponins, and steroids. CRE extract gave excellent MIC (Minimum Inhibitory Concentration) effects against pathogenic bacteria and fungal strains. CRE had significant results against *Bacillus thruengenesis* with 1.25 mg MIC, OD value of sample was 0.01±0.0003 (±SD) with 0.0101-0.0122 range and with *Candida vaginitis* (fungal strains) with 6.25 mg, OD value of sample was 0.04±0.0001 (±SD) with 0.0421-0.0429 range. CRE extract had given more than 85% inhibition against Colon cancer cell lines (DLD-1), therefore, this was better than positive control used i.e. Doxorubicine antibiotic (85.74%). CRE also gave significant results i.e. >70% against breast cell lines (MCF-7), Breast metastatic cell line (MDMAMB-468). So, out of six experimented cell lines, CRE gave very good inhibition for three cell lines i.e. Breast (MCF-7), breast metastatic (MDMAMB-468) and Colon cancer cell lines (DLD-1).

Conclusion CRE extract had shown the significant antimicrobial and antifungal effect, against *Bacillus threngenesis* and *Candida vaginitis* respectively. CRE also showed an excellent anticancer effect against Breast cancer cell line (MCF-7), Breast metastatic cell line (MDMAMB-468) and Colon cell line (DLD-1). -

Keywords: Calotropis procera, Anticancer, Antimicrobial, CRE, Roots, Cell lines, In-vitro, etc.

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INTRODUCTION

Even now, 80 % of people in the developing countries depend upon traditional medicines. Over 40 % of all pharmaceuticals available in the USA depend upon natural sources [3]. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs of higher efficacy or as models for pharmacologically active compounds [4]. There are several standard methods used for the phytochemical screening of medicinal plants as described for alkaloids, steroids, phenols, flavonoids, saponins, glycosides and tannins [5]. Methods for quantitative analysis of phytochemicals [phenolics, flavonoids, alkaloid, saponins and glycosides) were also developed [3].

Bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents [6-9]. Efforts are still on to search for effective naturally occurring anticarcinogens that would prevent, slow or reverse cancer development. The root bark and leaves of Calotropis procera are used by various tribes of central India as a curative agent for jaundice [10]. The plants have been reported to passes diverse biological activities. Different parts of the plant have been found to passes proteolytic, antimicrobial, larvicidal and anticancer activities. Calotropis procera is small, erect and compact shrub, which has been known to possess analgesic antitumor, antihelmintic, antioxidant, hepatoprotective, antidiarrheal, anticonvulsant, antimicrobial, estrogenic, antinociceptive and antimalarial activity [11]. Ethanol has been shown as a best solvent to yield, extracted component and antimicrobial effect. In CRE and CSE extracts, only root extracts have been shown best inhibitory effects on all concerned microbes [12].

Considering several pharmacological properties of Calotropis procera, we prepared to examine anticancer, antimicrobial and antifungal. Therefore, the present study was designed to screen traditionally used Calotropis procera for their immuno-property against anticancer, pathogenic bacteria and fungal strains.

MATERIALS AND METHODS

Methodology and used chemical details may be followed by the relevant article [13].

RESULTS AND DISCUSSION

Phytochemical estimation of *Calotropis procera* roots ethanol extract:

In our study, out of seven secondary metabolites, three secondary metabolites (i.e. alkaloids, saponins, and steroids) were present in the Calotropis root ethanol extract by using phytochemical estimation process (Table-1). Abegunde et al [14] were also extracted the Alkaloid, glycosides, saponin, terpenoid and carbohydrate from the Calotropis procera root, stem and leaf ethanol extract [14]. The Calotropis root ethanol extract has also shown the presence of alkaloids, resins, saponins, reducing sugar, steroids and tannins [17]. Whereas some other previous studies reported that steroids, flavonoids and tannins have their potential for inhibition, it may be possible due to their quality to react with protein and form stable (water soluble) compounds by damaging the cell membrane of microbial strains [15-16].

Table 1: Phytochemical estimation of Calotropis procera roots ethanol extract.

S.No.	Secondary Metabolites	Method	Results
1	**Alkaloid**	Hager's method and Tannic acid	Positive
2	**Saponin**	Foam test	Positive
3	**Steroid**	Salkowaski reaction	Positive
4	**Flavonoid**	Shindona test and Sodium hydroxide test	Negative
5	**Tannin**	Lead Acetate test	Negative
6	**Cd. Glycoside**	Kellar Killani test and Baljet's test	Negative
7	**Anthroquinone**	Confirmation test from kokate (Pharmacology Hand Book)	Negative

In present study, antimicrobial activity on *E. coli* shows that stem and root of *Calotropis procera* with ethanol extract given highest inhibition and selected for further analysis. *Calotropis procera* root ethanol extract have given excellent results against all bacterial strains like *Salmonella typhi, Chlamydia pneumonia, Bacillus thruengenesis, Pseudomonas aeruginosa,* and *Cornybacterium diphtheria.* CRE had excellent results against *Bacillus thruengenesis* with 1.25 mg

MIC, OD value 0.01±0.0003 (±SD) and 0.0101-0.0122 range (Table-2 and figure 1.1-1.5). MIC against fungal strains Candida vaginitis with 6.25 mg, OD value of sample was 0.04±0.0001 (±SD) and 0.0421-0.0429 range (Table-3 and figure 1.6-1.10). CRE extracts showing best results for all selected strains The effect exhibited by ethanolic extract of leaves and roots was significantly greater than the aqueous extract of leaves and roots [18]. A study demonstrates that the ethanolic extract of flowers of Calotropis procera (Asclepiadaceae) shows antibacterial action against four Gram positive micro-organisms, (Staphylococcus aureus, Bacillus subtilis, Bacillus pumilis, and Micrococcus lutes and Gram-Negative micro-organism (Escherichia coli, Pseudomonas aeruginosa, and Proteus vulgaris) [19].

Table 2: MIC of Calotropis procera roots ethanol extracts against bacteria strains.

S.No.	Name of Strains	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	Negative control	Positive control	MIC
1	Salmonella Typhi	0.01±0.0004 0.0103-0.0128	0.01±0.0002 0.0118-0.0132	0.03±0.0002 0.0312-0.0328	**0.02±0.0003** **0.0217-0.0233**	0.17±0.0002 0.1719-0.1732	**0.65**	**NG** **NG**	**2.5**
2	Chlamydia Pneumoniae	0±0.0002 0.0022-0.0038	0±0.0001 0.0002-0.0012	0.02±0.0004 0.0202-0.0231	**0.01±0.0005** **0.0118-0.0148**	0.22±0.0007 0.0003-0.2238	**0.61**	**NG** **NG**	**2.5**
3	Bacillus Thurengenesis	0±0.0003 0.0003-0.0021	0±0.0006 0-0.0038	0±0.0001 0.0001-0.0013	0.01±0.0005 0.0101-0.0129	**0.01±0.0003** **0.0101-0.0122**	**0.68**	**NG** **NG**	**1.25**
4	Pseudomonas aeruginosa	0±0.0003 0.0003-0.0021	0±0.0002 0.0005-0.0019	0.01±0.0002 0.0118-0.0132	**0.03±0.0005** **0.0311-0.0342**	0.02±0.0003 0.0207-0.0226	**0.61**	**NG** **NG**	**2.5**
5	Cornybacterium diphtheriae	0±0.0002 0.0001-0.0018	0±0.0003 0.0008-0.0027	**0.02±0.0004** **0.0211-0.0238**	0.25±0.0004 0.2510-0.2535	0.11±0.0003 0.1109-0.1131	**0.72**	**NG** **NG**	**5**

*±Standard Deviation with range, NG= No growth, MIC=Minimum Inhibitory Concentration, Negative control=DMSO, n=3

Table 3: MIC of Calotropis procera roots ethanol extract against fungal strains

S.No.	Name of strains	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.13 mg/ml	Negative control	Positive control	MIC
1	*Aspergillus fumigatus*	0.01±0.0008 0.0102-0.0148	0.01±0.0005 0.0118-0.0148	**0.05±0.0003** **0.0509-0.0528**	0.23±0.0054 0.2102-0.	0.45±0.0002 0.4509-0.4522	**0.43**	**NG**	**12.5**
2	*Cryptococcus neoformans*	0.02±0.0003 0.0208-0.0231	0.01±0.0003 0.0100-0.0121	**0.04±0.0002** **0.0403-0.0421**	0.19±0.0003 0.1909-0.1928	0.38±0.0003 03802-0.3822	**0.48**	**NG**	**12.5**
3	*Candida albicans*	0.02±0.0003 0.0209-0.0228	0.02±0.0006 0.0209-0.0244	**0.01±0.0007** **0.0088-0.0127**	0.18±0.0003 0.1802-0.1824	0.29±0.0005 0.0002-0.2942	**0.45**	**NG**	**12.5**
4	*Candida vaginitis*	0±0.0004 0.0002-0.0024	0±0.0002 0.0003-0.0013	0±0.0001 0.0003-0.0012	**0.04±0.0001** **0.0421-0.0429**	0.16±0.0002 0.1609-0.1621	**0.42**	**NG**	**6.25**
5	*Bacillus dermatitidis*	0.02±0.0008 0.0209-0.0254	0.04±0.0003 0.0403-0.0422	**0.04±0.0003** **0.0410-0.0432**	0.21±0.0002 0.2110-0.2121	0.43±0.0953 0.0426-0.4322	**0.48**	**NG**	**12.5**

*±Standard Deviation with range, NG= No growth, MIC=Minimum Inhibitory Concentration, Negative control=DMSO, n=3

TOTAL ACTIVITY OF CRE FOR BACTERIA AND FUNGI

CRE has shown highest total activity against *P. aeruginosa* gram +ve bacteria and minimal total activity against C. pneumonia gram –ve bacteria. So, CRE have capability to kill complete population of P. aeruginosa after 216-time dilution that is followed by B. thruengiensis and C. diphtheria (TA-190.58). Whereas, CRE may be diluted only 154 times and 129 times for C. S. typhi and pneumoniae respectively to inhibit the complete growth of the bacterial strains (Table-4 and Fig:2.1). Total activity of CRE on fungal strains was observed highest for C. vaginitis and C. neoformans that killed completely by the dilution of 26.34 and 26.55 respectively. However, CRE may be diluted as near as same dilution i.e. A. fumigates (24.17] and B. dermatitidis [24]. Whereas C. albicans need high concentration of CRE for its complete inhibition as compare to other experimented fungal strains (Table-5 and Fig: 2.2).

Table 4: Total activity of CRE for Bacteria

Pathogen details		Total Activity (TA)	
S.No	Strains	T A	MIC
1	*S. typhi*	154.28	2.1
2	*C. pneumoniae*	129.6	2.5
3	*B. thruengiensis*	190.58	1.7
4	*P. aeruginosa*	216	1.5
5	*C. diphtheria*	190.58	1.7

Table 5: Total activity of CRE for Fungi.

Pathogen details		Total Activity (TA)	
S.No	Strains	T A	MIC
1	*A. fumigatus*	24.17	13.4
2	*C. neoformans*	26.55	12.2
3	*C. albicans*	22.34	14.5
4	*C. vaginitis*	26.34	12.3
5	*B. dermatitidis*	24	13.5

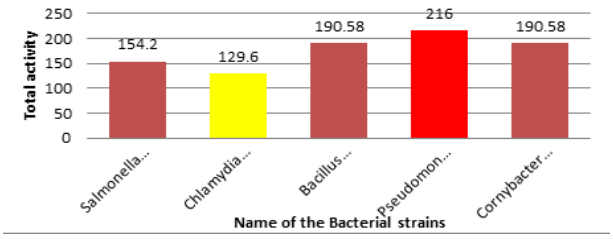


Figure 1: Total activity of CRE for bacterial and fungal strains presented in figure 2.1 and 2.2 as follows.

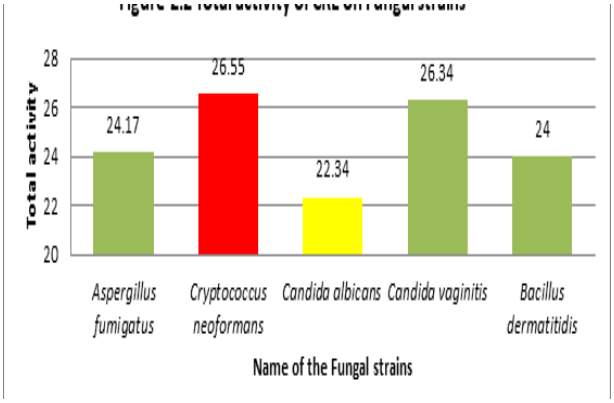
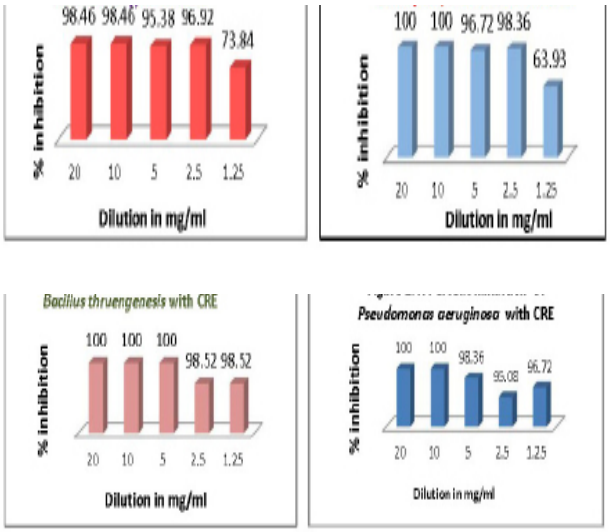


Figure 2: Calotropis root ethanol (CRE) extract percent inhibition activity against 5 bacterial and 5 fungal strains

The percent inhibition activity of CRE against selected bacterial cells found variation in the result (Fig-1.1-1.5). Percent inhibition of S. typhi was 96.92% (2.5 mg/ml), C. pneumoniae was 98.36% (2.5 mg/ml), B. thruengiensis was 98.52% (1.25 mg/ml), P. aeruginosa was 98.36% (2.5 mg/ml) and C. diphtheria was 98.60% (2.5 mg/ml). According to the percent inhibition analysis all selected bacterial strains given best sensitivity for CRE that given excellent percent inhibition against B. thruengiensis. Some previous studies demonstrated that the natural products of Calotropis procera have the antibacterial potential due to their basic character to react with proteins to form water soluble compounds, which can directly kill the bacteria by damaging its cell membrane [16, 18, 19]. In present study, CRE has been shown excellent inhibitory effect against A. fumigates 88.37% (12.5 mg/ml), C. neoformans 91.66% (12.5 mg/ml), C. albicans 90.77% (12.5 mg/ml), C. Vaginitis 90.47% (6.25 mg/ml) and B. dermatitidis 91.66% (12.5 mg/ml) Fig-1.6-1.10.



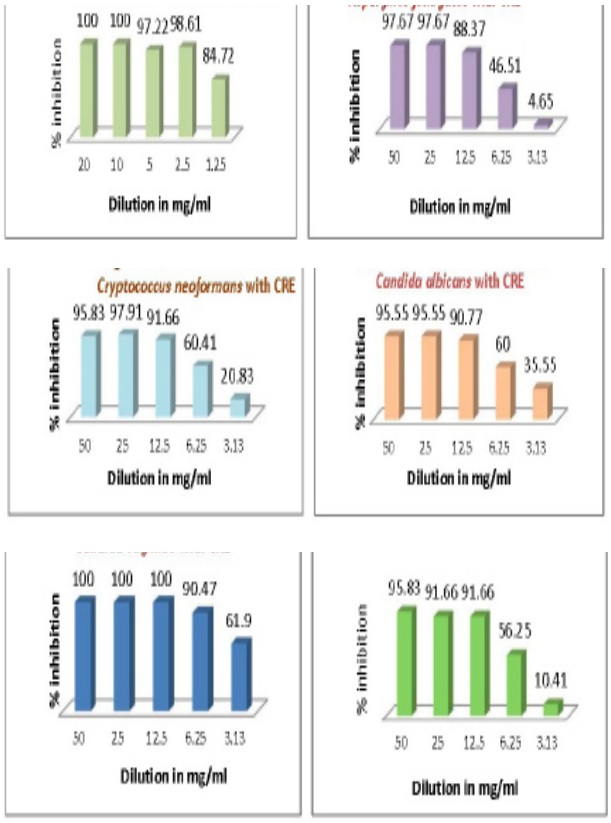


Figure 3: Calotropis root ethanol (CRE) extract percent inhibition activity against 5 bacterial and 5 fungal strains presented in figures 1.1 to 1.10 are as follows.

Percent Inhibition of anticancer effects of CRE and Doxorubicin drug against cancer and metastatic cancer cell lines

Calotropis root ethanol extract has selected for further analysis i.e. anticancer activity. CRE extract has very good effects against Breast (MCF-7), Breast metastatic (MDMAMB-468), Lung (A-549), Lung metastatic (NCIH358) and Colon metastatic (SW620). *Calotropis* root ethanol extract has more inhibitory effects than positive control (Doxorubicine) against Colon (DLD-1) cancer cell lines (Fig:3). Calotropis Dried Latex (Stem) treatment of mice showed a complete protection against hepatocarcinogenesis and alcoholic whole plant extracts of *Calotropis procera* and *Trigonella foenum graecum L.* showed in vitro cytotoxicity against different human cancer cell lines (21, 24) such as lung, neuroblastima, liver and colon by Sulforhodamine B dye (SRB) assay was done on cell lines namely of lung (A-549), liver (Hep-2), colon (502713, HT-29) and neuroblastima (IMR-32) (22-23). *Calotropis procera* ethanol and water extract of leaves and roots show anticancer activity. The root extract of *C. procera* has been found to produce a strong cytotoxic effect on COLO 320 tumor cells (24), our study also observed the same result against colon cell lines.

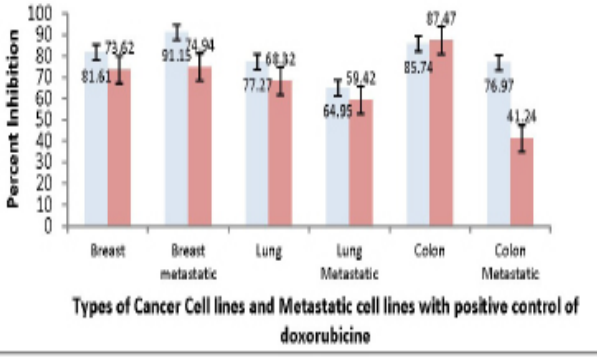


Figure 4: Percent Inhibition of anticancer effects of CRE and Doxorubicin drug against cancer and metastatic cancer cell lines

CONCLUSION

Calotropis procera root ethanol (CRE) extract gave very good results against all bacterial strains and fungal strains including cancer cell lines of Colon (DLD-1), Breast (MCF-7), Breast metastatic (MDMAMB-468). It was not reactive against colon metastatic cell lines (SW620), lung metastatic (NCIH358) and lung cancer cell lines (A549). The study would provide us future discernment in the evolution of ayurveda world and therapies for the treatment of various types of cancer and other diseases.

CONFLICT OF INTERESTS

The author(s) declare(s) that they have no conflict of interest to disclose.

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