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A REVIEW ON ANTIPROLIFERATIVE ACTIVITY OF PLANT EXTRACTS AGAINST BREAST CANCER CELL LINES

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ABSTRACT: Breast cancer (BC) is the foremost cause of deaths among women worldwide. Plants contain diverse bioactive phytochemicals which have been explored by researchers all over the world for their cancer preventive potential. The present review tabulates *in-vitro* tested plants during 2014-17 against breast cancer cell line. We have collected data of 56 angiosperm families (117 plant species) which was subjected to cluster analysis. On the bases of IC₅₀ values of plant extracts were clustered using cluster analysis Cluster analysis showed a grouping of order Brassicales, Fabales, Lamiales, Caryophyllales, Myrtales, and Apiales. It has been found that most of the plants tested against BC belong to eudicot group of plants. Active plant extract obtained after 24 h, 48 h and 72 h treatment were *Mimosa caesalpinnifolia*, *Ferulago angulat*, *Magydaris tomentosa*, and *Ipomea batatas* respectively. These plants may further be characterized for active ingredients to check their prospects in breast cancer treatment.

INTRODUCTION: Breast Cancer (BC) is the most prevalent cause of cancer-related deaths among women worldwide ^{1, 2, 3, 4, 5}. Despite advances in its diagnosis and treatment options, the number of incidences is increasing every year ^{6, 7}. The number of breast cancer cases reported in 2018 from the whole world was 20,88,849 amongst which 6,26,679 died ⁸. BC is not just one disease but has many variations and subtypes with distinct signatures and treatment programs ^{9, 10}.



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The early stage BC can be successfully cured, but treatment options are scarcely accessible to patients with advanced or metastatic stages ⁷. Women with mutated BRCA gene, have nearly 80% risk of developing BC along with a 50% possibility of their children getting the mutated gene 11. The genetic mutations in BRCA1 and BRCA2 genes suppurates the exigent root cause patrimonial breast cancer ¹². Although numerous causes are associated with the establishment and progression of BC, yet the oxidative stress (OS) is operating in most of the intracellular pathways concerned with cellular proliferation ¹³.

It has been reported repeatedly that the level of OS is higher for BC patients as compared to healthy people due to genetic abnormalities ^{1, 14}. This elevated OS is beneficial for malignant cells as it

upregulates the reactive oxygen species (ROS) mediated signaling pathways which encourage cell growth, cell differentiation, glucose synthesis, protein synthesis and hence cell survival. Numerous ROS are generated within the body as a result of basal metabolic activities. Hydrogen peroxide (H₂O₂) is one such ROS which is generated during estrogen metabolism, and it is known to activate extracellular regulated kinase 1/2 (Erk 1/2). Erk 1/2 is known to promote the survival of breast cancer cells in humans by activating downstream elements ¹⁵. Plant extracts being excellent scavengers of free radicals have often been implicated as a remedial measure in various diseases. The plant extracts contain numerous phytochemicals which act synergistically against disorders unlikely the purified compounds Numerous well established anticancer drugs in use today have been derived from plants such as Sulphoraphane, Paclitaxel, Epipodophyllotoxin, Vincristine, Vinblastin, Vinorelbine, Vindesine, Vinflunine, Pomiferin, Roscovitine, Flavopiridol, Noscapine 17, 18, 19. In light of the significant contribution of phytochemicals in cancer treatment, the present review was designed to compile the invitro antiproliferative activity of various plant extracts against breast cancer cell line.

2. MATERIALS AND METHODS:

2.1. Database Search: We have searched online free resource "PubMed" (maintained by National Center for Biotechnology Information at the National Library of Medicine, USA) for plants

extracts assessed for their antiproliferative activity against breast cancer cell line (MCF-7) employing *in-vitro* assays (MTT, MTS, XTT, CCK-8, SRB, CVS, WST-1, ATPlite, Alamar blue, Methylene blue, RTCA MP) for treatment period of 24, 48 and 72 h. The data was collected for the last four years *i.e.*, 2014-17.

- **2.2. Presentation of Collected Data:** The collected data was presented in the tabulated form. Various parameters selected for the present work were a plant, family, plant part used, the solvent used for extraction, assay employed and IC_{50} concentration.
- **2.3. Data Analysis:** Cluster analysis was done by using PAST software applying Ward's method, and Euclidian distance was calculated and presented as a measure of similarity.

3. RESULTS AND DISCUSSION:

- **3.1. Families and Orders:** The collected data covered 56angiosperm families and 118 plants **Table 1.** From these, 50 families belong to eudicots (22 orders), 4 families belong to monocots (4 orders), and 2 families belong to magnoliids (2 orders) as shown in **Table 2**.
- **3.2. Cluster Analysis (CA):** The CA was applied to IC₅₀ values of different plant species (as reported in the respective research paper) on the basis of above-ground plant part with treatment time of 24 (AG24), 48 (AG48) & 72 h (AG72); below ground with treatment period of 24 h (BG24).

TABLE 1: PLANT SPECIES TESTED AGAINST MCF-7 BREAST CANCER CELL LINE DURING 2014-18

S. no.	Family	Plant	Plant part	Solvent	Assay	Treatment	IC_{50}
						time (H)	(µg/ml)
1	Acanthaceae	Clinacanthus nutans ³²	Root	MeOH	MTT	24	35
		Avicennia alba ³³	Leaves	MeOH	MTT	48	117
		Ecbolium virde ³³	Leaves	MeOH	MTT	48	60
		Phlogacanthus thyrsiformis ³⁴	Leaves	EtOH	MTT	24	49
2	Anacardiaceae	Mangifera indica ³⁵	Kernal	EtOH	MTT	72	15
		Lannea coromandelica ³³	Bark	MeOH	MTT	48	270
			Leaves	MeOH	MTT	48	161
3	Apiaceae	Glehnia littoralis ³⁶	Root	AQ	CCK-8	24	158.63
		Ferula heuffelii ³⁷	Undergroun	CF	MTT	24	6.1
			d Parts	MeOH	MTT	24	47.6
4	Apocynaceae	Leptadenia reticulata ³⁸	Whole plant	AQ	MTT	24	740
		Picralima nitida ³⁹	Root Bark	EtOH	MTS	24	22.76
		Holarrhena floribunda ⁴⁰	Leaves	MeOH	MTT	24	357.6
						48	244.3
						72	126.7
		Hemidesmus indicus ⁴¹	Root	AQ	MTT	24	829.32

				30% EtOH	MTT	24	209.73
5	Araliaceae	Hedera nepalensis ⁴²	Aerial Part	MeOH+ CF	SRB	72	62
6	Asteraceae	Vernonia cinerea ⁴³	Whole plant	EtOH	SRB	72	60
		Gnaphalium luteoalbum ³³	Leaves	MeOH	MTT	48	340
		Artemisia indica ³⁴	Leaves	95% EtOH	MTT	24	48
		Launaea procumbens ⁴⁴	Leaves	MeOH	MTT	48	> 80
		Sphaeranthus	Whole plant	PE	MTT	48	23.55
		amaranthoide ⁴⁵	1				
		Anthemis mirheydari ⁴⁶	Whole plant	DCE	MTT	72	25.2
7	Berberidaceae	Berberis orthobotrys ³⁹	Root	MeOH	MTS	24	81.35
8	Bignoneaceae	Tabebuia impetigosa ⁴⁷	Dried inner	MeOH	SRB	24	110.76
	U	1 0	bark				
9	Cactaceae	Opuntia ficus-indica ⁴⁸	Stem	EA	MTT	48	138
10	Capparaceae	Crateva adansonii ⁴⁹	Stem Bark	DCM +	ABA	24	289
	11			MeOH			
11	Caprifoliaceae	Nardostachys jatamansi ⁵⁰	Roots +	MeOH	MTT	48	58.01
			Rhizome	PE	MTT	48	60.59
12	Caryophyllaceae	Arenaria montana ⁵¹	Aerial part	AQ	SRB	48	130.05
13	Chrysobalanaceae	Licania rigida ⁵²	Seeds	EtOH	ATPlite	24	N.C
	<i>j</i>	Licania tomentosa ⁵²	Seeds	EtOH	ATPlite	24	N.C
14	Combretaceae	Anogeissus latifolia ⁵³	Stem &	95% EtOH	MTT	48	20.1
			Leaves				
		Terminalia bellerica ⁵³	Stem &	95% EtOH	MTT	48	9
			Bark				
		Terminalia bellerica ⁵⁴	Fruits	70% MeOH	WST-1	48	104.65
		Terminalia chebula ⁵⁵	Leaf galls	EtOH	MTT	72	208.16
15	Convolvulaceae	Ipomoea batatas ⁵⁶	Peeled Root	Acidified	MTT	24	5.9
		F	tubers	EtOH			
			Not Peeled	Acidified	MTT	24	4.9
			Root tubers	EtOH			
16	Cucurbitaceae	Momordica	Aril	Hexane+	MTT	48	117
		cochinchinensis ⁵⁷		Acetone +			
				EtOH			
17	Dilleniaceae	Dillenia suffruticosa ⁵⁸	Root	DCE	MTT	24	20.3
		Dillenia suffruticosa ⁵⁹	Root	EA	MTT	24	76
		Dillenia indica ³³	Leaves	MeOH	MTT	48	340
18	Dioscoreaceae	Dioscorea villosa ⁶⁰	Root	MeOH	CC	72	27
19	Dipterocarpaceae	Dipterocarpus turbinatus ³³	Bark	MeOH	MTT	48	168
20	Droseraceae	Drsera burmanni ⁶¹	Whole plant	MeOH	WST-1	48	120.94
21	Ebenaceae	Diospyros peregrina ³³	Leaves	MeOH	MTT	48	7
22	Ericaceae	Vaccinium bracteatum ⁶²	Leaves	Hexane	RTCA	72	206.75
					MP		
23	Euphorbiaceae	Jatropha curcas ³⁹	Root Bark	EtOH	MTS	24	36.55
	•	Jatropha gossypifolia ³⁹	Root Bark	EtOH	MTS	24	25.55
		Croton sphaerogynus ⁶³	Leaves	EtOH	SRB	48	53.4
24	Fabaceae	Eythrina excelsa ⁶⁴	Stem Bark	EtOH	MTT	24	13.6
		Acacia catechu ⁵³	Fruit	50% EtOH	MTT	48	22.8
		Acacia catechu ⁵⁴	Heartwood	70% MtOH	MTT	48	288.85
		Enterolobium	Leaves	MeOH	MTT	48	11.84
		cyclocarpum ⁶⁵					
		Sophora interrupta ⁶⁶	Root	EA	MTT	24	250
		Caesalpinia pulcherrima ³³	Leaf	MeOH	MTT	48	240
		Clitoria ternatea ³³	Flower	MeOH	MTT	48	114
			Leaf	MeOH	MTT	48	170
		Alhagi graecorum ⁶⁷	Aerial Part	85% EtOH	CVA	48	36.4
		Saraca indica ⁶⁸	Bark	80% MtOH	MTT	72	73.6
		Pithecellobium dulce ¹⁶	Leaf	AQ	MTT	24	400
						48	300
		Mimosa caesalpiniifolia ⁶⁹	Leaves	70% EtOH	MTT	24	5
25	Geraniaceae	Pelargonium sidoides ⁷⁰	Root	AQ +	SRB	72	43

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44	Potenderiaceae	Eichhornia crassipes ¹⁰⁰	Whole	MeOH	SRB	72	1.2
			Plant				
45	Primulaceae	Aegiceras corniculatum ³³	Fruit	MeOH	MTT	48	91
		Maesa macrophylla ³⁴	Leaves	95% EtOH	MTT	24	22.66
46	Punicaceae	Punica granatum ¹⁰¹	Fruit	Fruit Juice	MTT	72	50
47	Resedaceae	Ochradenus arabicus ⁷⁴	Aerial part	95% EtOH	MTT	24	562
48	Rhamnaceae	Ziziphus spina-christi ¹⁰²	Leaves	80% EtOH	MTT	24	230
49	Rubiaceae	Hymenodictyon excelsum ³³	Bark	MeOH	MTT	48	80
			Wood	MeOH	MTT	48	72
		Mussaenda glabrata ³³	Leaves	MeOH	MTT	48	133
		Galium aparine ¹⁰³	Whole plant	MeOH	XTT	72	503
50	Rutaceae	Glycosmis pentaphylla ¹⁰⁴	Leaves	PE	SRB	48	95.5
51	Salicaceae	Casearia sylvestris ¹⁰⁵	Leaves	AQ EtOH	MTT	24	141
52	Solanaceae	Capsicum annuum ¹⁰⁶	Seeds	AQ	CPC	120	14.7
53	Thymelaeaceae	Phaleria macrocarpa ¹⁰⁷	Fruit	AQ MeOH	MTT	24	96
			Seed	AQ MeOH	MTT	24	12
54	Urticaceae	Urtica pilulifera ⁷⁵	Aerial part	EtOH	MTT	24	63
55	Verbenaceae	Lantana camara ¹⁰⁸	Whole plant	EtOH	MTT	24	32.39
		Clerodendrum viscosum ³³	Leaves	MeOH	MTT	48	50
56	Zingiberaceae	Curcuma zedoaria ¹⁰⁹	Rhizome	Hexane	MTT	72	18.4
		Etlingera elatior ¹¹⁰	Flowers	Water +	MTT	72	173.1
		-		EtOH			
		Alpinia galanga ¹¹¹	Rhizome	EtOH	MTT	72	170
		Curcuma kwangsiensis ¹¹²	Rhizome	HD	MTT	24	82.3
N. G. N.				DOE D		nn n 1	

N.C = Not Cytotoxic; MeOH = Methanol; EtOH = Ethanol; DCM = Dichloromethane; DCE = Dichloroethane; PE = Petroleum ether; EA = Ethyl Acetate; ABA = Alamar Blue Assay; CC = Cell Counting; CVA = Crystal Violet Assay; MBA = Methylene Blue Assay; CPC = Coulter particle counter; AQ = Aqueous; CF = Chloroform; HD = Hydrodistillation; CP = Cold Pressing; CPC = Coulter particle counter; MBA = Methylene blue assay.

TABLE 2: NUMBER OF ORDERS AND FAMILIES OF PLANT SPECIES (TESTED AGAINST BREAST CANCER CELL LINE) BELONGING TO VARIOUS APG CLADES

S. no.	APG Clade	No. of orders	No. of families	No. of plants
1	Eudicots	22	50	108
2	Monocots	04	04	07
3	Magnoliids	02	02	03

3.2.1. CA of AG24: AG24 involved 31 plant species belonging to 23 families. The CA of AG24 revealed a clustering of *Crateva adansonii* (Capparaceae) with *Moringa oliferna* (Moringaceae) both belonging to order Brassicales and *Mimosa caesalpiniifolia* with *Eythrina excelsa* which belong to family Fabaceae and order Fabales **Fig. 1.** The minimum IC₅₀ value was found in *Momosa caesalpiniifolia* (5.0 μg/ml) while maximum IC₅₀ in *Leptadenia reticulata* (740 μg/ml).

3.2.2. CA of AG48: AG48 involved 44 plants belonging to 31 families. The CA of AG48 showed clustering of *Clerodendrum viscosum* (Verbenaceae) with *Melissa officinalis* (Lamiaceae), both belonging to order Lamiales. Clustering was also observed in plants of Caryophyllales order *i.e, Opuntia ficus-indica* (Cactaceae) with *Arenaria montana* (Caryophyllaceae) **Fig. 2**. The minimum IC₅₀ value was found in *Ferulago angulata* (5.3 μg/ml) while maximum IC₅₀ in *Syzygium aromaticum* (455 μg/ml).

3.2.3. CA of AG72: The AG72 group had 27 plants which were spread across 16 families. The CA presented aggregation of families belonging to order Lamiales and Myrtales. The plants belonging to order lamiales were *Stachys persica*, *Stachys pubescens* and *Stachys byzantinai*.

Order Myrtales included *Pimenta dioica* and *Punica granatum* **Fig. 3**. The minimum IC_{50} value was found in *Magydaris tomentosa* (0.94 µg/ml) while maximum IC_{50} in *Galium aparins* (503 µg/ml).

3.2.4. CA of BG24: The BG24 group included 13 plants belonging to 10 families. The CA showed grouping of *Glehnia littotalis* and *Hemidesmus indicus* belonging to family Apiaceae and order Apiales **Fig. 4**.

The minimum IC_{50} value was found in *Ipomoea batatas* (5.9 µg/ml) while maximum IC_{50} in *Sophora interrupta* (250 µg/ml).

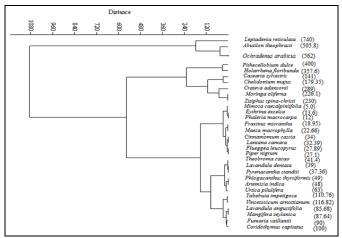


FIG. 1: CLUSTER ANALYSIS OF ABOVE GROUND PLANT PARTS WITH TREATMENT PERIOD OF 24 h (AG24) ALONG WITH THEIR IC₅₀ VALUE (µg/ml)

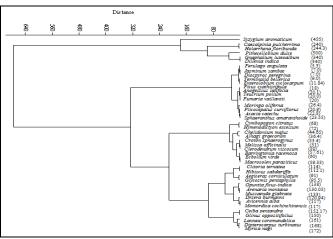


FIG. 2: CLUSTER ANALYSIS OF ABOVE GROUND PLANT PARTS WITH TREATMENT PERIOD OF 48 h (AG48) ALONG WITH THEIR IC 50 VALUE (µg/ml)

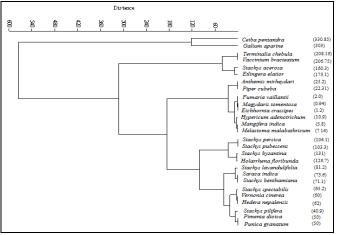


FIG. 3: CLUSTER ANALYSIS OF ABOVE GROUND PLANT PARTS WITH TREATMENT PERIOD OF 72 h (AG72) ALONG WITH THEIR IC50 VALUE (μ g/ml)

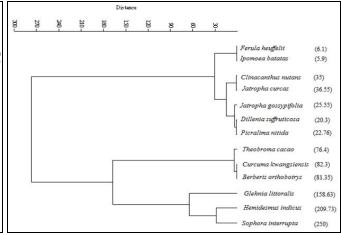


FIG. 4: CLUSTER ANALYSIS OF BELOW GROUND PLANT PARTS WITH TREATMENT PERIOD of 24 h (BG24) ALONG WITH THEIR IC₅₀ VALUE (µg/ml)

3.3. Assay Reported: The *in-vitro* tetrazolium and resazurin-based reduction assays employed by authors were MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide); CCK-8 (Cell counting kit-8); MTS (3-(4,5-dimethylthiazol-2-yl)-5- (3-carboxymethoxyphenyl)- 2- (4-sulfophenyl)-2H-tetrazolium, inner salt); XTT (2,3-bis(2methoxy-4-nitro-5-sulphophenyl)-5-carboxanilide-2H-tetrazolium, monosodium salt): SRB (Sulforhodamine B colorimetric assay); ABA (Alamar blue assay); WST (2-(4-iodophenyl)-3-(4nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt); Luminogenic ATP (Adenosine triphosphate); RTCA-MP (real-time impedance-based cell growth method); CVA (Crystal violet staining).

3.4. Causes of Breast Cancer: There are numerous causes of breast cancers as conversed in proceeding discussion. Elderly women are at more

risk of developing breast cancer as compared to younger women ²⁰. Post-menopausal women develop a greater threat of having breast cancer, which doubles with every passing decade to 80 years of life ²¹. The risk of getting breast cancer is reduced by bearing a child. This has been justified by the lower rates of incidences in married women as compared to single women ²². The reason may be early differentiation of mammary stem cells which belittles the threat of developing breast cancer ²³.

Mutations in certain high penetrance genes like RCA1, BRCA2, PTEN, TP53, CDH1, and STK11and lower penetrance genes (CHEK2, BRIP1, ATM, and PALB2) are responsible for breast cancer incidences ^{24, 25}. Long term or frequent exposure to polycyclic aromatic hydrocarbons (PAHs) disrupt estrogen metabolism and induce mammary cancer ²⁶.

Most of the ovarian hormones taken after menopause to allay its effects increase the rate of breast cancer induction in postmenopausal women ²⁷. Increased alcohol consumption in women is also linked with breast cancer incidences ²⁸. Lanky lifestyle like persistent obesity and unhealthy

dietary intake is associated with breast cancer ²⁹.

Exposure to ionizing radiations especially during breast development elevates the risk of developing breast cancer which can be avoided by lessening repetitive needless testing ^{29, 30}. Lopsided work schedule of women in developing as well as developed nations have also been positively correlated to increased breast cancer incidences ³¹. **Table 3** and **Fig. 5** compares the number of breast cancer cases reported their percentage of mortality in different countries.

TABLE 3: BREAST CANCER INCIDENCES REPORTED AND PERCENTAGE of CANCER DEATHS IN DIFFERENT COUNTRIES (GLOBOCAN 2014)

2014)			
Country	Population	Breast	% of breast
		cancer	cancer
			death cases
India	124×10^{7}	144937	21.5
Bangladesh	155×10^{6}	14836	16.9
Afgganistan	29825000	3108	22.8
Australia	23050000	14710	17.3
China	139×10^{7}	187213	-
France	63937000	54245	19.9
Germany	82800000	71623	18.8
Italy	60885000	50658	18.2
Japan	1.27×10^{6}	55710	9.2
Pakistan	1.79×10^{6}	34038	30.8
South Africa	52386000	9815	16
Sri Lanka	21098000	3955	18.8
USA	318×10^{6}	232714	16.1

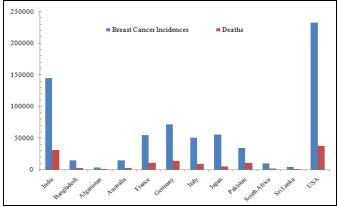


FIG. 5: NUMBER OF BREAST CANCER PATIENTS REPORTED AND PERCENTAGE CAUSALITIES IN DIFFERENT COUNTRIES

CONCLUSION: It has been concluded that most of the plants tested against breast cancer cell line belong to eudicots. Above ground plant parts showed better antiproliferative activity as compared to below ground plant parts. Active plant extract obtained after 24 h, 48 h and 72 h treatment were *Mimosa caesalpinnifolia*, *Ferulago angulat*, *Magydaris tomentosa*, and *Ipomea batats*. These plants must be characterized for active ingredients which can further be used *in-vivo* studies in animals induced with breast cancer to find out their prospects in breast cancer treatment.

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