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ANTIOXIDANT, ANTIMICROBIAL AND ANTIPROLIFERATIVE ACTIVITIES OF LEAF EXTRACTS OF THE INDIAN TRADITIONAL MEDICINAL PLANT *WRIGHTIA ARBOREA*

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Keywords:

Antioxidant, Antimicrobial, Phytochemistry, Anticancer, *Wrightia arborea*

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ABSTRACT: Introduction: *Wrightia arborea* (Apocynaceae) has been used in traditional medicine to treat ailments such as menstrual and renal complaints, snake-bites, tooth-ache and diarrhea. **Objective:** This study aimed to evaluate the phytochemical constituents, antioxidant, antimicrobial and antiproliferative activities of *W. arborea* leaf extracts. **Materials and Methods**: Total phenolic and flavonoid contents of the leaf extracts were determined. The antioxidant activity of the extracts were investigated using DPPH, ABTS, nitric oxide scavenging assays, phosphomolybdenum assay, reducing power and metal chelating assay. Antimicrobial activity was tested against MTCC strains and MDR clinical bacteria. The antiproliferative activity was determined using MTT assay against three cancer cell lines. **Results**: Extracts showed high total phenolic content ranging from 85.7± 1.15 to 40.3± 0.6 gallic acid equivalents and flavonoid content ranging from 750±5.5 to 140±3.6 quercetin equivalents per gram of dry extract. Ethyl acetate extract showed highest DPPH scavenging activity with an EC_{50} value of 330µg/ml. The antimicrobial effects of all the extracts were found to be genus specific - only against *Klebsiella spp.* The chloroform extract showed antiproliferative activity against K562 cells following 24 h exposure with an IC_{50} value of 40±3µg/ml which progressively decreased to 26±2µg/ml and 5µg/ml on prolonged exposure at 48 and 72 h respectively. **Conclusion**: High levels of total phenolics, flavonoids, antioxidants and selective antimicrobial and antiproliferative activities of *W. arborea* leaf extracts have been detected in our study. This first report validates its use in traditional medicine and as a potent bioresource for neutraceuticals and therapeutics.

INTRODUCTION: Plants represent a rich bioresource of natural antioxidants[1]. The commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ) are suspected to be toxic and carcinogenic [2, 3]. Hence, the demand for natural antioxidants has been on the increase since they have also been shown to confer several benefits to human health by scavenging free radicals and neutralizing the oxidative damage caused by them [4, 5, 6].

Overproduction and long-term exposure to free radicals such as superoxide anion, hydroxyl, nitroxide, peroxynitrite, singlet molecular oxygen and hydrogen peroxide produced during aerobic cellular metabolism are known to damage lipids, proteins and DNA [7] causing irreversible cellular dysfunction. Almost all traditional medicinal systems including Ayurveda use plants as their primary source of medicinal preparations providing

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valuable information on their therapeutic potential, which needs to be authenticated by modern research [8, 9].

The genus *Wrightia*, native to tropical Africa, Asia and Australia comprises of 32 flowering plants in the Apocynaceae family. Extracts prepared from various parts of *Wrightia* plants have been shown to possess a wide range of bioactivities including anticancer, antimicrobial, amoebicidal, analgesic and anti-inflammatory. Most of the studies on *Wrightia* species have been conducted mainly on *W. tinctoria* and *W. tomentosa*. While the former is widely used in Siddha system of medicine for treatment of psoriasis and other skin diseases the latter has been used in traditional medicine for renal complications, menstrual disorders, amoebic dysentery, fever and cancer. Many phytoconstituents such as triterpenoids, flavonoids, triterpenes isolated from different parts of *W. tinctoria* have been reported. Cytotoxic alkaloids known as Wrightiamines A and B have also been reported from another species called *W. javanica* [10, 11].

W. arborea, a lesser studied member of the genus *Wrightia*, is a small tree or shrub chiefly seen in the Indian deciduous forests located in Deccan - Carnatic region, Sri Lanka, Burma and Thailand. This plant has been used in Ayurveda, Siddha and other traditional systems of medicine. The bark is known to possess antipyretic and antibacterial activities and is also used for curing menstrual and renal complaints. The latex of this plant is used as an antidote for snake bites and scorpion-stings. The leaves are used as a diaphoretic, expectorant and to treat dysentery, toothache and diarrhea. The alcoholic extracts of the leaves possess remarkable analgesic, anti-inflammatory and wound healing activities. Barring a single report on the properties of methanolic extracts from leaf [10] *W. arborea* remains virtually unexplored. Thus further detailed investigations should prove useful in providing a better understanding of the pharmacological action of the plant.

Hence, the present study was undertaken to evaluate the antioxidant, antimicrobial and antiproliferative activities of different organic solvent extracts of *W. arborea* leaves.

MATERIALS AND METHODS:

Chemicals: Folin-Ciocalteau's reagent, 2,2-azino-bis (3 - ethylbenz - thiazoline - 6 - sulphonicacid) (ABTS) substrate solution, sodium nitroprusside, ferrozine, MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide and dimethyl suphoxide (DMSO) were purchased from SRL Pvt. Ltd., Mumbai, India. 1, 1 – diphenyl - 2-picrylhydrazyl (DPPH), peroxidase, Roswell Park Memorial Iinstitute medium (RPMI-1640), Dulbecco's Modified Eagle's Medium (DMEM) were obtained from HiMedia Laboratories, Mumbai, India. p-iodonitrotetrazolium chloride was purchased from Sigma - Aldrich, USA. Fetal bovine serum (FBS) was procured from Gibco, USA. All other chemicals and reagents used were of analytical grade.

Plant samples: Leaves of *W. arborea* were collected from the Botanical garden of Calicut University in July 2013 and authenticated at the Department of Botany, University of Calicut. A voucher specimen (Voucher No #6833) has been deposited in the Calicut University Herbarium.

Preparation of Extracts: Shade-dried leaves were coarsely powdered (20 g) and subjected to defatting by refluxing with 100ml of petroleum ether at 60-80^{O}C for 12 h. This was followed by successive solvent extractions by a process of continuous soxhlation. The extractions were done with individual solvents (100 ml) possessing increasing polarities such as chloroform, acetone, ethyl acetate and methanol. The crude extracts obtained were filtered through Whatman filter paper No.1. Following evaporation of the solvents, the resultant residues were dissolved in DMSO to get a stock solution of 20 mg/ml. The extracts were designated as WAA, WAC, WAE and WAM with the first two letters denoting *W. arborea* and the third denoting acetone, chloroform, ethyl acetate and methanol respectively.

Estimation of total phenolics: Total phenolic content in each extract of *W. arborea* was determined by Folin-Ciocalteau method using gallic acid as standard [12]. Briefly, 200 µl of Folin-Ciocalteau's reagent was added to 2.0 ml of each extract and kept for 10 min at room temperature. To this, 300 µl of 15% Na_2CO_3 was added.

The mixtures were allowed to stand at 25^{O}C for 2 h and absorbance was measured at 765 nm using a UV-visible spectrophotometer (UV-1601, Shimadzu, Japan). The total phenolic content of the extracts were expressed as gallic acid equivalents (GAE).

Estimation of total flavonoids: The total flavonoid contents of *W. arborea* leaf extracts were determined as described by Fu et al. [13]. Briefly, 20 µl of each extract (1 mg/ml) was mixed with 30 µl of 5 % sodium nitrite. After 6 min, 50 µl of 10% aluminum chloride was added, and the mixture was allowed to stand for a further 5 min. Then, to the above mixture, 100 µl of 10% NaOH was added and its absorbance was measured after 15 min at 510 nm. The total flavonoid content of each of the extracts was expressed as Quercetin equivalents (QE).

Antioxidant activities:

DPPH radical method: The DPPH radical scavenging activity of the extracts was evaluated by the method of Huang et al. [14] using ascorbic acid as positive control. To 1.0 ml of each extract, at concentrations ranging from 0.2 to 1 mg/ml, an equal volume of 100 mM methanolic DPPH was added. The mixture was then incubated in dark for 30 min at 25^{O}C followed by absorbance measurements at 517 nm. The activity was determined using the formula:

[(absorbance of control – absorbance of sample) / absorbance of control] X100.

ABTS scavenging activity: The antioxidant capacity of samples was measured by a modified protocol as described by Chen et al. [15]. Equal volumes (1.0 ml) of peroxidase (4.4 units/ml), H_2O_2 (50 µM), (ABTS) substrate solution (100 µM) and distilled water were mixed and kept in dark for 1 h. To this mixture, 1.0 ml of plant extract was subsequently added and absorbance was recorded at 734 nm. The antioxidant capacity was calculated by the following formula:

[1− (absorbance of sample/ absorbance of control)] × 100

Nitric oxide scavenging activity: Nitric oxide scavenging activity was estimated by Griess reagent assay [16]. Briefly, 2.0 ml of 10 mM sodium nitroprusside was mixed with 0.5 ml phosphate buffered saline (PBS) and 0.5 ml of each extract at varying concentrations. The mixture was then incubated at 25°C for 2.5 h. To 0.5 ml of the reaction mixture, 1.0 ml sulphanilic acid reagent (0.33% sulphanilic acid in 20% glacial acetic acid) was added and allowed to stand for 5 min followed by addition of 1.0 ml of 0.1% naphthyl ethylene diamine dihydrochloride. This incubation mixture was allowed to stand for 30 min followed by recording of its absorbance at 540 nm. Ascorbic acid was used as positive control. Percentage scavenging was calculated by the following formula:

[(absorbance of control – absorbance of sample)/ absorbance of control] × 100

Determination of reducing power: To 1.0 ml of each plant extract at varying concentrations (0.2-1 mg/ml), 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium hexacyanoferrate (1% w/v) were added and the mixture was incubated at 50^{O}C in a water bath for 20 min. After cooling to room temperature, 0.5 ml of trichloroacetic acid (10% w/v) was added followed by centrifugation at 3000g for 10 min. To 1.0 ml of the supernatant, an equal volume of distilled water and 0.1 ml of freshly prepared 1.0% ferric chloride solution were added. Absorbance of the mixture was read at 700 nm. Ascorbic acid was used as standard [12].

Phosphomolybdenum assay: The total antioxidant capacity of various extracts of *W. arborea* was evaluated using the phosphomolybdenum method with minor modifications [17]. Different concentrations of the extracts (0.2 -1.0 mg/ml) were dissolved in 3.0 ml of reagent solution mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the solution was measured at 695 nm against a reagent blank comprising the respective solvents. The results have been reported as ascorbic acid equivalents.

Metal chelating activity: The chelation of ferrous ions by extracts was estimated by the method of

Dinis et al [18]. Briefly, 50 µl of 2 mM $FeCl_2$ was added to 1.0 ml of the extract at different concentrations (0.2 - 1.0 mg/ml) followed by the addition of 0.2 ml of 5 mM ferrozine solution. After vigorous shaking the mixture was left to stand at room temperature for 10 min. The absorbance of the solution was thereafter recorded at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula:

[(absorbance of control - absorbance of the extract)/ absorbance of the extract] x100

Antimicrobial activity:

Microorganisms: Bacterial strains used in this study – a) MTCC strains - *E. coli* 41, *Pseudomonas aeruginosa* 424, *Klebsiella pneumonia* 3384, *Proteus mirabilis* 425, *Acinetobacter boumanii* 1920 and *Staphylococcus aureus* 87 were purchased from Institute of Microbial Technology, Chandigarh, India and b) multidrug resistant clinical bacterial isolates designated as - *Klebsiella MDR, Staphylococcus MDR, Pseudomonas MDR, E. coli MDR, Acinetobacter MDR* and *Proteus MDR* were collected from various hospitals in Kerala State, India.

Determination of antibacterial activity: All bacterial isolates were tested against *W. arborea* extracts, by using the Kirby-Bauer agar well - diffusion method [19]. Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculum on the surface of the media. Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5.0 ml of nutrient broth and incubated at 37^{o} C for 3-5 h till a moderate turbidity was developed.

The turbidity was matched with 0.5 McFarland standards. Wells, 8 mm in diameter, were punched in the agar and 75 µl of each extract was added to the peripheral wells with DMSO alone serving as a negative control in the central well. The plates were incubated at 37^{o} C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition in millimetres.

Determination of minimal inhibitory concentration (MIC):

Microbroth dilution method: MIC values of extracts against tested microorganisms were determined by a modified serial dilution microplate bioassay using specific dye p-iodonitrotetrazolium chloride as an indicator of growth[20]. About 100 µl of Muller Hinton broth culture of bacteria (10^6 CFU/ml) was inoculated into each well of a microtitre plate followed by the addition of 100 µl of each extract at different concentrations. The microplates were incubated overnight at 37°C followed by addition of 40 µl of 0.2 mg/ml p-iodonitrotetrazolium solution to each well. After 1 h of incubation, the MIC values - the lowest extract concentration at which bacterial growth was inhibited - were recorded.

Antiproliferative activity:

Cells and culture conditions: The cytotoxicity was tested against three human cancer cell lines - chronic myelogenous leukemic K562 cells, breast cancer MCF-7 cells and hepatic carcinoma Hep G2cells. K562 cells were cultured in RPMI-1640 medium while MCF-7 and Hep G2 cells were cultured in DMEM. Both media were supplemented with FBS (10% v/v), streptomycin (100µg/ml) and penicillin (100U/ml) and maintained in an incubator at 37°C in a humidified, 5% CO_2 atmosphere.

MTT Assay: To evaluate the effect of plant extracts on the proliferation of three cell lines, standard MTT assay was performed. K562 ($2x10^4$ cells/well), MCF- 7 and Hep G2 cells (7000 cells/ well) were seeded into 96 well plates and cultured overnight. The cells were then treated with varying concentrations of different extracts of *W. arborea*. Cells treated with equivalent amounts of solvent DMSO were used as negative control. After incubating for a period of 24, 48 and 72 h, MTT (500 µg/ml) was added to each well and incubated at 37^{o}C for 3 h. The medium was discarded and 150 µl DMSO was added into each well at room temperature for 10 min with shaking. The absorbance was measured at 570 nm on a plate reader (Multiscan EX, Thermo scientific, USA) and the difference of absorbance between the treated and untreated control groups was used to determine cell viability [21].

Statistical analysis: All results were expressed as the mean ± standard deviation (SD) from three independent experiments. The data were analyzed by ANOVA procedures. A post-hoc test (LSD) was carried out when the differences shown by the data were significant ($p < 0.05$). SPSS (version 15.0) statistical program was used for all analysis.

RESULTS AND DISCUSSION:

Phytochemical analysis:

Extract Yield, total phenolics and flavonoid content: The percentage yield (*w/w*) of each of the four different extracts of *W. arborea* ranged from 5.0215 to 0.6575 and was found to be in the order of: WAM > WAA>WAE > WAC (**Table 1**). The highest total phenolic content of 85.7±1.15 mg (GAE/g dry weight of the extract) was recorded in WAM and the lowest, 40.3±0.6mg in WAE ($p < 0.05$). The values were found to decrease in the order of WAM > WAC > WAA > WAE. The highest total flavonoid content was observed in WAC (750±5.5mg QE /g dry weight of the extract), a fivefold enriched value when compared to the lowest (140±3.6) found in WAE. The flavonoid content was found to decrease in the order of WAC > WAA > WAM >WAE.

TABLE 1 YIELD, QUANTITATIVE PROFILE OF TOTAL FLAVONOID AND PHENOLIC CONTENT IN THE DIFFERENT EXTRACTS OF *W. ARBOREA*.

Extract	Yield (% w/w)	Total flavonoid content (mg) (Quercetin equivalent/g extract)	Total phenolic content (mg) (Gallic acid equivalent/g extract)
WAA	1.741	278 ± 3.8	58.7± 2
WAC	0.6575	750 ± 5.5	61.3± 1
WAE	1.06	140 ± 3.6	40.3± 0.6
WAM	5.0215	249 ± 3.2	85.7± 1.15

WAA - *W. arborea* acetone, WAC - *W. arborea* chloroform, WAE - *W. arborea* ethyl acetate, WAM - *W. arborea* methanol. Each value represent mean ± S.D. (n=3).

Antioxidant Assays:

DPPH radical scavenging activity: DPPH is a stable free radical. It reacts with free radical scavengers yielding a stable product - 1, 1-diphenyl-2-picrylhydrazine – showing a color change from purple to yellow. In this study all the extracts showed DPPH scavenging activities in a dose-dependent manner which varied among different extracts. The DPPH scavenging activity of different extracts of *W. arborea* is shown in **Fig. 1**. The highest DPPH scavenging activity was shown by WAE extract with an EC_{50} value of 330 µg/ml while the lowest was observed in WAC extract with an EC_{50} value of 950 µg/ml ($p < 0.05$); the activities decreased in the order of WAE>WAM>WAA>WAC (**Fig.1).**

ABTS scavenging Activity: ABTS (2, 2'-azinobis- (3-ethylbenzothiazoline-6-sulphonic acid) is converted to its radical cation by reacting with H_2O_2 in the presence of peroxidase. The radical cation is blue in colour and absorbs light at 734 nm. The ABTS radical cation reacts with antioxidant and its blue colour is converted back to colourless neutral form. In the present study all the different extracts of *W. arborea* showed ABTS scavenging activity in the order of WAE>WAM>WAC>WAA ($p < 0.05$, **Fig.1**).

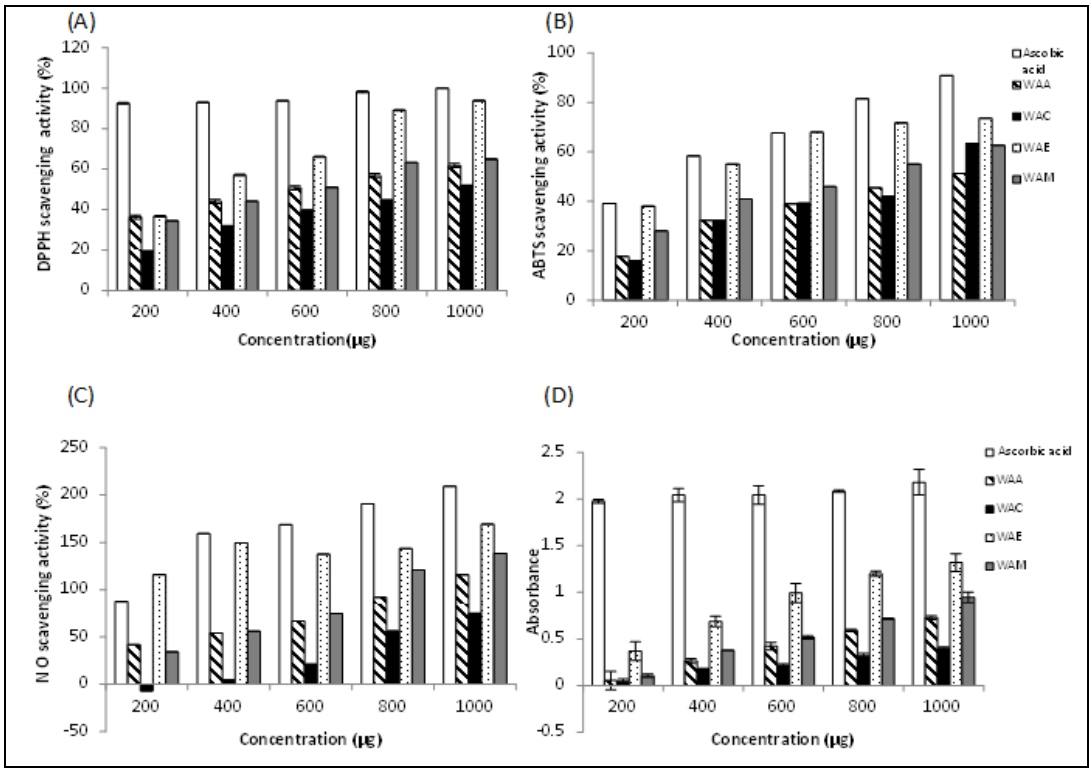
Nitric Oxide scavenging activity: In aqueous solutions sodium nitroprusside spontaneously generate nitric oxides under physiological conditions. On contact with oxygen, nitric oxide produces nitrite ions which react with Griess reagent to form a purple azodye. In the present study all the different extracts of *W. arborea* showed nitric oxide scavenging activity in the order of WAE>WAM>WAA>WAC ($p < 0.05$, **Fig. 1**).

Reducing power: The antioxidant activity was also determined by assessing the reducing power of the different extracts using a modified Fe^{3+} to Fe^{2+} reduction assay. Antioxidants cause reduction of Fe^{3+}-Ferricyanide complex to Fe^{2+} form which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. A dose-dependent increase in the reducing power of the different extracts was observed and the activity was found to decrease in the order of WAE> WAM> WAA> WAC ($p < 0.05$, **Fig.1**).

Phosphomolybdenum assay: The total antioxidant activity of different extracts of *W. arborea* was measured by phosphomolybdenum assay. The antioxidants convert Mo (VI) to Mo (V) and form a green phosphate / Mo (V) compound with an absorption maxima at 695 nm. The result expressed as number of equivalents of ascorbic acid is presented in **Table 2**. The total antioxidant activity of the different extracts of *W. arborea* was found to decrease in the order of WAE> WAA = WAM> WAC at a concentration of 1.0 mg/ml ($p < 0.05$).

TABLE 2: TOTAL ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *W. ARBOREA*. VALUES REPRESENT MEAN ± S.D. OF THREE EXPERIMENTS.

Extract	Total antioxidant activity
WAA	57.3±2.516mg/AAE
WAC	45.6±3.05 mg/AAE
WAE	170±2.645 mg/AAE
WAM	57±2.645mg/AAE



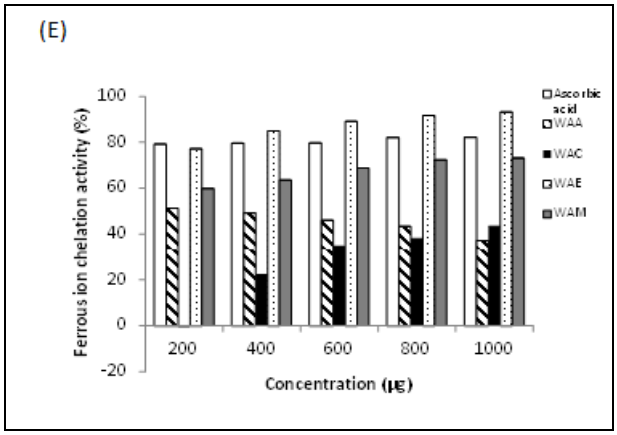
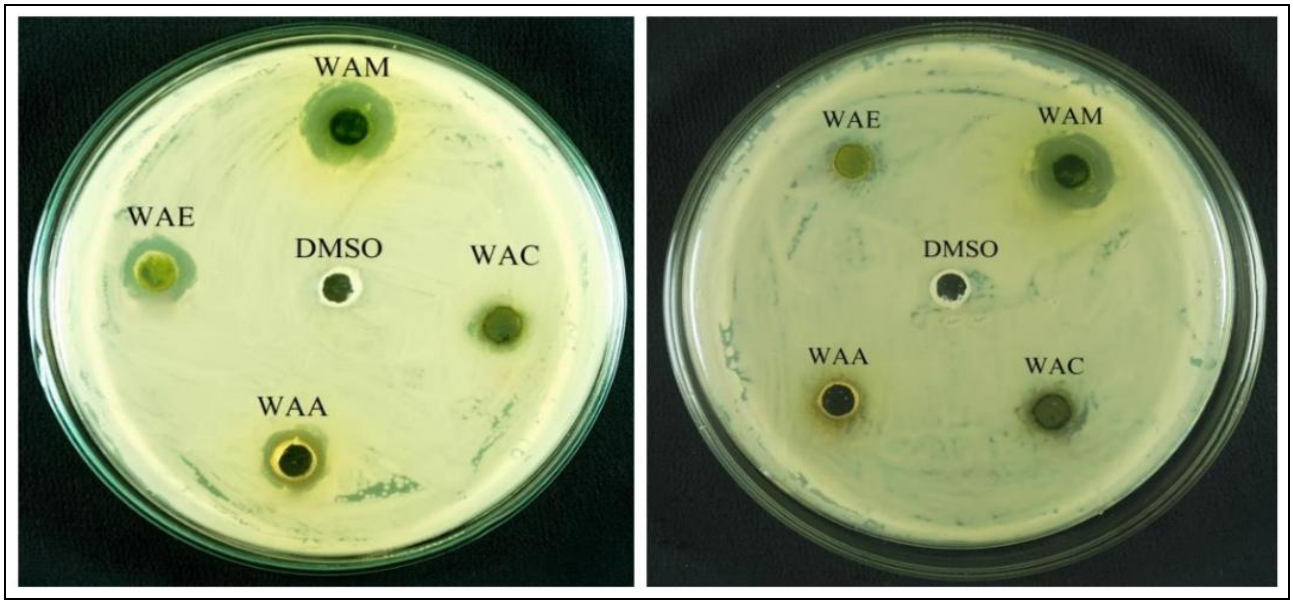


FIG. 1: ANTIOXIDANT ACTIVITIES OF DIFFERENT EXTRACTS OF *W. ARBOREA* (A) DPPH scavenging activity (B) ABTS scavenging activity (C) Nitric oxide scavenging activity (D) Reducing power assay (E) Ferrous ion chelation activity. WAA- *W. arborea* acetone, WAC- *W. arborea* chloroform, WAE- *W. arborea* ethyl acetate, WAM - *W. arborea* methanol. Values represent mean±S.D. of three experiments.

Antimicrobial activity: *In vitro* antimicrobial activities of the four extracts of *W. arborea* were tested against each of the six MTCC and multidrug resistant bacteria by agar well diffusion assay (**Fig. 2**) and the MIC values were calculated by microbroth dilution method (**Table 3**). Our results showed that all of the extracts possessed specific antimicrobial activity only against *Klebsiella pneumonia* MTCC 3384 strain. The maximum zone of inhibition was obtained with WAM followed by that of WAE, WAA and WAC respectively. Strikingly, among the multidrug resistant bacterial strains tested, only *Klebsiella* MDR strain was found to be sensitive to WAM. It is interesting to note that all the extracts showed a genus specific effect, even though to different extents. Intriguingly, this genus specificity, however, was also evident in multidrug resistant strains tested, one of which was found to be sensitive only to WAM but not to the other extracts.



KLEBSIELLA MTCC 3384 **KLEBSIELLA MDR**

FIG. 2: ZONE OF INHIBITION SHOWN BY *KLEBSIELLA* STRAINS (MTCC AND MDR) AGAINST DIFFERENT *W. ARBOREA* LEAF EXTRACTS. WAA - *W. arborea* acetone, WAC - *W. arborea* chloroform, WAE - *W. arborea ethyl acetate*, WAM - *W. arborea* methanol, DMSO – Dimethylsuphoxide used as the solvent vehicle served as control. Values represent mean±S.D. (n=3).

TABLE 3: THE MIC VALUES (mg/ml) OF DIFFERENT *W.ARBOREA* EXTRACTS.

Microorganism	Plant extracts (mg/ml)			
	Acetone	Chloroform	Ethyl Acetate	Methanol
Klebsiella pneumonia 3384	5.2±0.3	5.8±0.3	4.2±0.3	3.2±.03
Klebsiella MDR	-	-	-	6. 2±0.3

Values represent mean ± S.D. of three experiments.

Cell proliferation Assay: The cytotoxic properties of different extracts of *W. arborea* on K562, MCF-7 and Hep G2 cells were evaluated using MTT assay. The cells were exposed to the extracts for 24, 48 and 72 h duration. The results are shown in **Fig. 3.** The highest IC_{50} value of 40±3 µg /ml was obtained with WAC treatment for 24 h on K562 cells which was found decreased after 48 h treatment to 26±2 µg/ml plummeting further down to 5µg/ml after 72 h in a concentration and time-dependent manner ($p < 0.05$). As evidenced by the IC_{50} values, all extracts except WAC, however, showed negligible activity on all of the three cell types tested. Unlike the drastic decrease in IC_{50} values obtained following WAC treatment in K562 cells mentioned above, the IC_{50} values in the case of Hep G2 cells were found to be stable with >105±3 µg/ml, 103±1 µg/ml and 100±3 µg/ml following 24, 48 and 72 h treatment respectively ($p < 0.05$). However, in MCF-7 the IC_{50} values obtained with WAC were 96±3 µg/ml, 99±1 µg/ml and 105±3 µg/ml for 24, 48 and 72 h respectively ($p<0.05$).

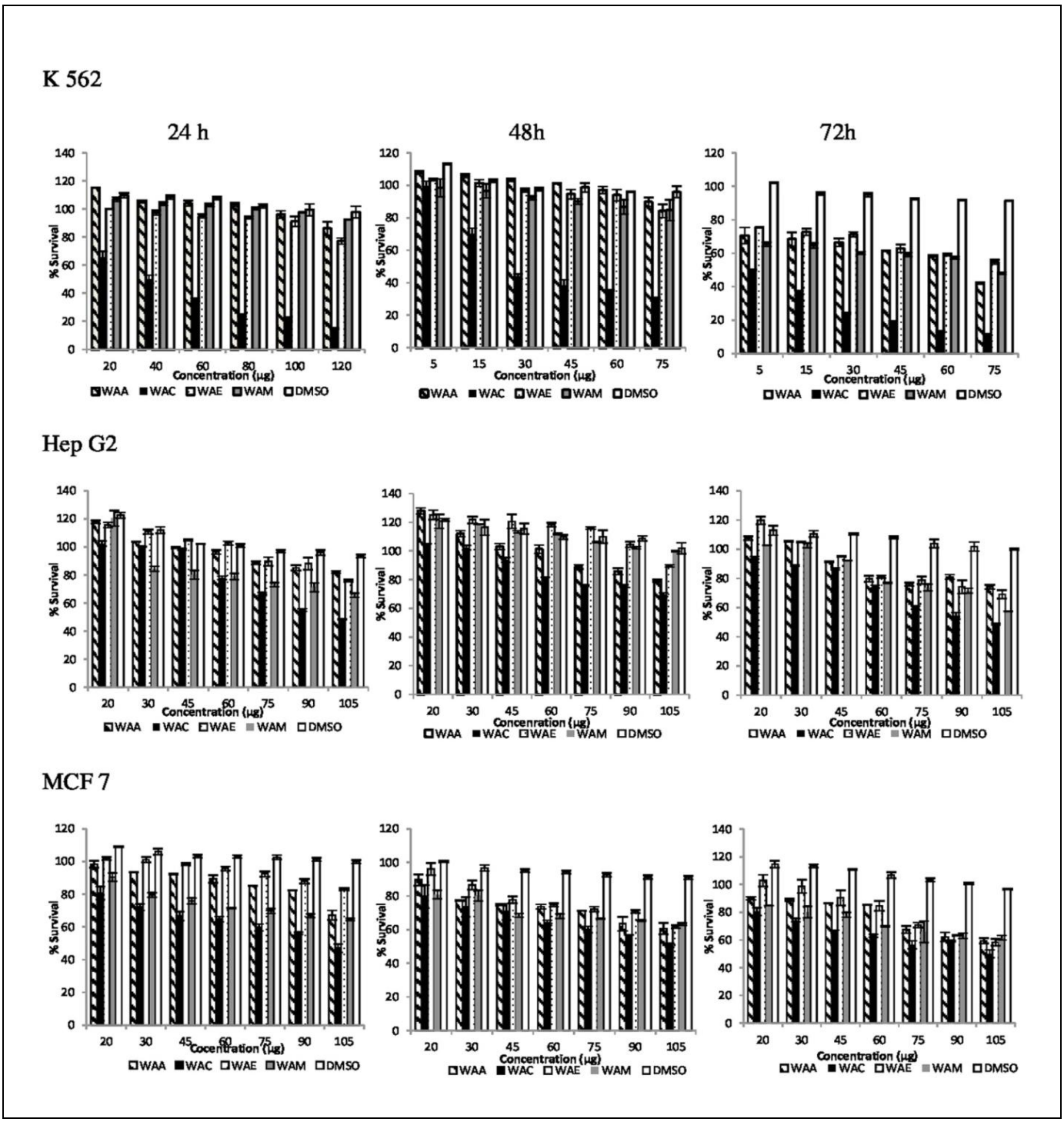


FIG. 3: ANTIPROLIFERATIVE ACTIVITIES OF *W. ARBOREA* EXTRACTS ON CANCER CELL LINES – K562, HEP G2 AND MCF 7 AFTER 24, 48 AND 72 H TREATMENTS. WAA - *W. arborea* acetone, WAC - *W. arborea* chloroform, WAE - *W. arborea* ethyl acetate, WAM - *W. arborea* methanol, DMSO – Dimethylsuphoxide used as the solvent vehicle served as control. Values represent mean±S.D. (n=3).

DISCUSSION: Reactive oxygen species (ROS) such as superoxide anion (O^{2-}), hydroxyl radical (HO^{-}) and hydrogen peroxide (H_2O_2) are physiological metabolites generated during aerobic life as a result of the metabolism of oxygen [22, 23]. These ROS cause irreversible damage to the components of a cell, such as lipids, proteins and DNA[24]. Production of free radicals in the biological system results in oxidative stress, which occurs due to an imbalance between the production of free radicals and antioxidant defences [12, 25]. Antioxidants fight against free radicals and protect humans from various diseases. They exert their action either by scavenging the reactive oxygen

species or by protecting the antioxidant defense mechanisms.

The antioxidant activity of the extracts was analyzed by six different assays which include DPPH scavenging activity, ABTS scavenging activity, Nitric oxide scavenging activity, Reducing power assay, Ferric ion scavenging assay and Phosphomolybdenum assay. The antioxidant activity of biological samples varied depending on the assay used. Interestingly, irrespective of the different assays performed, our results showed that the extracts exhibited a similar pattern of antioxidant potential as follows -WAE> WAM> WAA> WAC.

Among the various natural antioxidants, constituents such as phenolics and flavonoids are of prime importance due to their multiple biological effects and direct contribution to antioxidative activity [26]. The results of our study reveal that there is no apparent correlation between antioxidant activity and phenolic content. Methanolic extracts obtained from *W. arborea* leaves presented a higher phenolic content than that of other solvent extracts. This fact correlates with the remarkable differences in the polarity of the extraction solvents used and solubility of phenolic compounds in them. Methanol (polar solvent) is considered as one of the best solvents for phenolics extraction [27].

Antibacterial activity of all the four extracts of *Wrightia arborea* was assayed. The methanol extract showed specific antimicrobial activity only against *Klebsiella* species.

Our results showed that there was no apparent correlation between the antioxidant and antiproliferative activities [28] in the extracts tested. This experiment suggests that the inhibition of tumor cell proliferation in vitro by the extracts of *W. arborea* cannot be solely explained on the basis of the concentration of phenolic /flavonoid compounds. The inhibition of cancer cell proliferation may be attributed to some unknown compound(s) present in the *W. arborea* leaf extracts.

CONCLUSION: The present investigation revealed that all of the four organic extracts prepared from *W. arborea* leaves possessed significantly high levels of total phenolic and flavonoid content. Although all extracts showed antioxidant potential, ethyl acetate extract was found to possess the highest activity. Interestingly, the methanol extract showed genus-specific antibacterial activity against *Klebsiella* among the various MTCC and MDR bacterial strains tested. The chloroform extract, which incidentally exhibited a fivefold enriched flavonoid content compared to ethyl acetate extract, showed antiproliferative activity only against human leukemic K562 cells among the three cell lines tested. Further investigations are justified towards identifying the active components and their mode of action for future clinical drug development.

Conflict of interest: The authors declare no conflict of interests.

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REFERENCES:

1. Gan RU, Kuang L, Xu XR, Zhang Y, Xia EQ, Song FL, Li HB. Screening of natural antioxidants from traditional Chinese medicinal plants associated with treatment of Rheumatic Disease. Molecules. 2010,15:5988-5997.
2. Nieva-Echevarria B, Manzanos MJ, Goicoechea E, Guillen MD. 2,6-Di-Tert-Butyl-Hydroxy toluene and its metabolites in foods. Compr rev food sci food saf. 2015, 14:67-80.
3. Schmidt H, Stocker R, Vollbracht C, Paulsen G, Riley D, Daiber A, Cuadrado A. Antioxidants in translational medicine. Antiox redox signal. 2015, doi: 10.1089/ars.2015.6393.
4. Wojcik M, Burzynska-Pedziwiatr I, Wozniak LA. A review of natural and synthetic antioxidants important for health and longevity. Curr Med Chem. 2010, 17:3262-3288.
5. Xu YJ, Tappia PS, Neki NS, Dhalla NS. Prevention of Diabetes–induced cardiovascular complications upon treatment with antioxidants. Heart fails rev. 2014, 19:113-121.
6. Shah NA, Khan MR, Ahmad B, Noureen F, Rashid U, Khan RA. Investigation on flavonoid composition and anti free radical potential of *Sida cordata;* BMC Medicine.2013, 13:276.
7. Katiyar DK, Mehrotra A, Pant KK. Hypoglycemic and antioxidant effects of a polyherbal formulation in alloxan induced diabetic rats. Ann Ayurvedic Med. 2012, 1:134-139.
8. Bazylko A, Piwowarski JP, Filipek A, Bonarewicz J, Tomczyk M. In vitro antioxidant and anti inflammatory activities of extracts from *Potentilla recta* and its main ellagitannin, agrimoniin. J Ethnopharmacol. 2013, 149:222–227.

9. Pandey MM, Rastogi S, Rawat AKS. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evid based complement alternat med. 2013, doi.org/10.1155/2013/376327.
10. Zahan R, Nahar L, Mosaddik A, Rashid MA, Hassan A, Ahmed M. Evaluation of antioxidant and antitumor activities of *Wrightia arborea*. J Basic Appl. Sci. 2013, 9:625-632.
11. Nahar L, Nasrin F, Zahan R, Mossadik MA. Antinoiceptive and anti inflammatory activities of *W.arborea.* Pak J Biol Sci. 2013,16:485-490.
12. Rajan I, Rabindran R, Jayasreee PR, Kumar PRM. Antioxidant potential and oxidative DNA damage preventive activity of unexplored endemic species of Curcuma. Indian J Exp Bio. 2014,52:133-138.
13. Fu R, Zhang T, Guo YR, Huang QL, Peng T, Xu Y, TangL, Chen F. Antioxidant and anti-inflammatory activities of the phenolic extracts of *Sapium sebiferum* (L) Roxb.leaves. J of Ethno pharmacol. 2013, 147:517–524.
14. Huang B, Ban X, He J, Tong J, Tian, J, Wang YW. Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (*Nelumbo nucifera* Gaertn.) leaves. Food Chem 2010, 120: 873–878.
15. Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Food Hum Nutr. 2008,63:15-20.
16. Chaudhary S, Chandrashekhar KS, Pai KSR, Setty MM, Devkar RA, Reddy ND, Shoja MH. Evaluation of antioxidant and anticancer activity of extract and fractions of *Nardostachys jatamansi* DC in breast carcinoma. BMC Complement Altern Med. 2015, 15:50.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application for the determination of Vit E. Anal. Biochem. 1999, 269:337-341.
18. Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994,315:161-169.
19. Bauer AW, Perry DM, Kirby WM. Single disc antibiotic sensitivity testing of *Staphylococci*. Arch Int Med. 1959, 104:208-216.
20. Eloff JN. A Sensitive and Quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998, 64:711-713.
21. Mosmann T. Rapid colorimentric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J imm methods. 1983,65:55-63.
22. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems and apoptosis. Free Radik Biol Med 2010, 48: 749-762.
23. Hayyan M,Hshim MA, AlNashef IM. Superoxide ion : generation and chemical implications. Chem. Rev 2016, 116: 3029-3085
24. Duan Y, Gao Y, Zhang J, Chen Y, Jiang Y, Ji J, Zhang J, Chen X, Yang Q, Su L, Zhang J, Liu B, Zhu Z, Wang L, Yu Y. Mitochondrial aldehyde dehydrogenase 2 protects gastric mucosa cells against DNA damage caused by oxidative stress. Free Radik Biol Med 2016, 93:165-176.
25. Poljsak B, Suput D, Milisav I. Achieving the balance between ROS and antioxidant: when to use the synthetic antioxidants. Oxid Med Cell Longev 2013, http://dx.doi.org/10.1155/2013/956792
26. Dai J, Mumper RJ. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 2001, 15: 7313-7352.
27. Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, Jeronimo C, Silva BM. Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L 2010, Food Chem Toxicol 48:441–447.
28. Lin ES, Chou HJ, Kuo PL, Huang YC.Antioxidant and antiproliferative activities of methanolic extract of Perilla frutescens. J Med Plants Res 2010, 4:477-483.

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