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Determination of Antioxidant and Antimicrobial Potential of Some Myanmar Medicinal Plants

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Abstract: **Some selected medicinal plants used by Myanmar people were evaluated for potential antioxidant and antimicrobial properties. In this study, the antioxidant activity of 14 types of medicinal plants collected from Kyaukse origin of Mandalay Division in the country Myanmar was estimated using1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ferric reducing antioxidant power (FRAP) assay and total phenol content assay. The highest antioxidant activity was demonstrated by *Vitis vinifer Linn*. *Phyllanthus urinaria* and *Zingiber officinale* in all tested *in vitro* antioxidant assays. As far as antimicrobial activity is concerned, ethanolic extracts of *Vitis vinifer Linn, Sida acuta, Phyllanthus urinaria , Z. officinale Calendula officinalis Caccinia Indica* and *Curcuma Longa* were found to be most potent against clinically important bacteria *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Enterococcus faecalis*. Brine shrimp lethality test was also conducted to examine the toxicity of selected plant extracts. The experimental results suggest that all the tested plant extracts are non-toxic and no potential health risk for human consumption.**

Keywords: **Medicinal plants, Myanmar, Antioxidant, Antimicrobial activity, Toxicity.**

I. INTRODUCTION

The oxidative stress, defined as "the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage" has been suggested to be the cause of aging and various diseases in humans. In modern western medicine, the balance between anti-oxidation and oxidation is believed to be a critical concept maintaining a healthy biological system. [1, 2] Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and can thus prevent or repair the damage done to the body's cells by oxygen [3].

Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources [4]. Some of the in vivo free radicals play a positive role in phagocytosis, energy production and regulation of cell growth etc. However, free radicals may also be damaging. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources [5]. Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals [6]. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases [7]. Epidemiological and in vitro studies on medicinal

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plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. [8, 9]

On continuation of our screening project for the search of antioxidant activity of popular medicinal plants in Myanmar, we studied 14 medicinal plant extracts. Total antioxidant potential has been determined using ferric reducing antioxidant power assay. The efficiency of extracted phenolic was evaluated using total phenol content assay. The free radical scavenging activity against 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) of all plant extracts were studied in this report.

Furthermore, plants promise a source of natural antimicrobial agents. It has been reported that the antimicrobial activity of plants is related with the defense mechanism against microorganism. [10]So, the antimicrobial activity of selected plant extracts against clinically important bacteria *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Enterococcus faecalis* are also tested.

II. MATERIALS AND METHODS

A. Preparation of Plant extracts:

Fourteen medicinal plant materials were collected from Kyaukse area in Mandalay Division. The plant materials were cleaned, air-dried for 5-7 days and cut into small pieces. And then, each piece of sample (100g) was extracted with 70% ethanol 400ml for about eight hours by Reflux (hot extraction method) and then filtered with filter papers. After filtration, the filtrates were concentrated by using distillation method. The concentrated plant extracts were dried and evaporated in air for a few days. The botanical names, family name, English name, parts used are presented in Table 1.

Table 1. Characteristics of the used medicinal plants

No	Botanical Name	English Name	Family Name	Parts Used
1	*Vitis vinifer* Linn.	Grapes	Vitaceae	Seeds
2	*Sida acuta*	Common wireweed	Malvaceae	The whole plants
3	*Clausena excavata*	Pink lime-berry	Rutaceae	leaves
4	*Phyllanthus urinaria*	Chamber bitter	Euphorbiaceae	The whole plants
5	*Thespespesia populnea*	Portia tree	Malvaceae	Leaves
6	*Achiyranthes aspera*	Prickly chaff-flower	Amaranthaceae	The whole plants
7	*Zingiber officinale*	Ginger	Zingiberaceae	Rhizomes
8	*Calendula officinalis*	Calendula	Compositae	The whole plants
9	*Citrullus vulgaris*	Water melon	Cucurbitaceae	Fruits
10	*Horde Vulgare*	Barley	Gramineae	The Whole plants
11	*Coccinia Indica*	Ivy gourd	Cucurbitaceae	Aerial parts
12	*Curcuma Longa*	Tumeric	Zingiberaceae	Rhizomes
13	*Salanum xanthocarpum* Linn.	Yellow Berried Night shade	Salanaceae	The whole plants
14	*Allium cepa* Linn.	Onion	Liliaceae	The whole plants

B. Preliminary Phytochemical Examination of Plant Samples:

Preliminary Phytochemical Examination of the selected plant samples were also carried out by the investigation of the organic constituents such as alkaloid, glycoside, reducing sugar, phenolic compound, flavonoid, saponin glycoside, cyanogenic glycoside, amino acid, carbohydrate, acid or base or neutral, tannin, steroid and terpenoid according to the test standard.

C. Examination for Natural Toxin of Crude plant extracts by using brine shrimp toxicity Test:

Toxicity test was carried out according to the method of Teng Wah Sam (1993). [11]

1ml of each of the serially diluted test solutions(6 tested samples) were added into separated virals. Ten nauplii were collected with Pasteur pipette from the hatching jar and transferred to each viral containing diluted test solutions. Potassium dichromate was kept as positive control and ethanol and natural sea water as negative controls. The bottles were capped with aluminum foil and stored in the dark room while the temperature was controlled at 25±1°C.

$$\text{DPPH Scavenged (\%)} = (A_{control} - A_{test} / A_{control}) \times 100$$

After incubation in the dark room, the virals were taken out and counted all tested larvae. The lethal concentration for 50% mortality (LC_{50}) after 24 hours of exposure. The same procedure using potassium dichromate served as positive control was conducted. All the experiments were performed in duplicate.

D. Determination of Antioxidant Activity:

1). Free radical scavenging ability by the use of a stable DPPH radical (1, 1-diphenyl-2-picryl-hydrazyl):

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method(TEKAO et al. , 1994) adopted with suitable modifications [12]. The stock solution of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62. 5, 40, 20, 10, 5 and 2. 5 µg/ml. Diluted solutions(1ml each) were mixed with 1 ml of methonolic solution of DPPH in concentration of 1mg/ml. After 30 min incubation in darkness at room temperature(23° C), the absorbance was recorded at 700 nm. The DPPH with corresponding solvents (without plant material) serves as the control. The methanol with respective plant extracts serves as blank. Methanolic solutions of pure compounds Vitamin C(L-ascorbic acid) were tested too at different concentrations(x mol of antioxidant/ 1mol of DPPH radical).

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh(1994)[13]

Where $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of the tested samples.

2) Determination of total phenolic contents in the plant extracts:

The concentration of phenolics in plant extracts was determined using spectrophotometric method(Singleton et al. , 1999)[14]. Methanolic solution of the extract in the concentration of 1mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0. 5 ml of methanolic solutionof extract, 2. 5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2. 4 ml 7. 5% $NaHCO_3$, Blank was concomitantly prepared, containing 0. 5 ml methanol, 2. 5 ml 10% Folin-Ciocalteu reagent dissolved in water and 2. 5 ml of 7. 5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was red (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

3) Measurement of reducing power:

The extracts were taken in different concentrations in phosphate buffer(0. 2mol/L, pH 6. 6) and incubated with potassium ferricyanide (1g/100ml water) at 50°C for 20 min. The reaction was terminated by adding TCA solution (10g/100ml water), centrifuged at 3000rpm for 10 min and the supernatant was mixed with ferric chloride(0. 1g/100 ml water). The absorbances measured at 700nm. The samples were prepared in triplicate for each analysis and the men value of absorbance was obtained. The increased absorbance of the reaction mixture indicated increased reducing power.

4) Statistical analysis:

Samples were analyzed in triplicate and the results were given as Mean ± S. D.

E. Detection of Antimicrobial Activity:

The antimicrobial activity was measured by Agar well diffusion assay. The tested microorganisms are *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Enterococcus faecalis.* Petri plates containing

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20ml Muller Hinton medium were seeded with the bacterial strains. Each labeled medium plate was uniformly inoculated with a test organism by using sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. Wells were punchered(8mm in diameter) and 12. 5mg/25 µl of ethanolic plant extracts were added. The plates were then incubated at 37°C for 24 hours. Ampicillin(10µg/25µl) was used as positive control and analysis was done in duplicates. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

III. RESULT AND DISCUSSION

A. Preliminary Phytochemical Examination of Plant Samples:

According to the result of preliminary phytochemical test, it shows that it may be safe for further research. These plant samples reveal the presence of phenolic and flavonoid compounds which are related to antioxidant activity as shown in Table(2)

Table(2) Preliminary Phytochemical Test of Selected Myanmar Medicinal Plants

Test Medicinal plants	Alkaloid	Glycoside	Carbohydrate	Reducing sugar	Saponin glycoside	Tannin	Flavonoid	Acid or Base	Phenolic compound	Amino acid	Cyanogenic glycoside
Vitis vinifer Linn.	+	+	-	+	+	+	+	Neutral	+	+	-
Sida acuta	+	+	+	+	+	+	+	Base	+	+	-
Clausena excavata	+	+	-	+	+	+	+	Neutral	+	+	-
Phyllanthus urinaria	+	+	-	+	+	+	+	Neutral	+	+	-
Thespespesia populnea	+	+	-	+	+	+	+	Neutral	+	+	-
Achiyranthes aspera	+	+	-	+	+	+	+	Neutral	+	+	-
Zingiber officinale	+	+	+	-	+	-	-	Neutral	-	+	-
Calendula officinalis	+	+	-	+	+	+	+	Neutral	+	+	-
Citrullus vulgaris	+	+	-	+	+	+	+	Neutral	+	+	-
Horde Vulgare	+	+	-	+	+	+	+	Base	+	+	-
Coccinia Indica	+	+	-	-	+	-	-	Base	+	+	-
Curcuma Longa	+	+	-	+	+	+	+	Base	-	-	-
Salanum xanthocarpum Linn.	+	+	-	+	+	+	+	Base	+	+	-
Allium cepa Linn.	+	+	+	+	+	-	-	Acid	-	-	-

+ = present, - = absence

B. Examination for Natural Toxin of Crude plant extracts by using brine shrimp toxicity Test:

Brine shrimp toxicity test is a preliminary toxicity screening that enables determination of medium lethal concentraton(LC_{50}) values for the extracts. Based on the results obtained, the respective extracts showed nontoxic, indicating that the samples are biologically active.

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Table(3) Brine shrimp toxicity expressed as the LC_{50} values of the medicinal plant extracts

No.	Botanical Name	LC50 (µg/ml) ± S. D
1.	*Vitis vinifer Linn.*	1462 ± 1. 29
2.	*Sida acuta*	607. 02 ± 1. 25
3.	*Clausena excavata*	199. 4 ± 1. 14
4.	*Phyllanthus urinaria*	2143 ± 1. 22
5.	*Thespespesia populnea*	3985. 02 ± 1. 1
6.	*Achiyranthes aspera*	450. 56 ± 1. 25
7.	*Zingiber officinale*	119. 74 ± 1. 2
8.	*Calendula officinalis*	1230 ± 1. 28
9.	*Citrullus vulgaris*	1248. 59 ± 1. 27
10.	*Horde Vulgare*	2442. 87 ± 1. 12
11.	*Caccinia Indica*	1054. 99 ± 1. 14
12.	*Curcuma Longa*	1481. 49 ± 1. 17
13.	*Salanum xanthocarpum Linn.*	1625. 74 ± 1. 12
14.	*Allium cepa Linn.*	861. 39±1. 12
15.	Potassium dichromate(K2Cr2O7)	14 ± 2. 13

C. Determination of Antioxidant Activity:

Extracts are subjected for the evaluation of antioxidant activity by using various in vitro model systems. DPPH radical scavenging ability of 14 medicinal plants were screened in ethanol solvent system as shown in figure 1. *Vitis vinifer Linn* seed (IC_{50} value=5. 0042) showed strong inhibition of DPPH radical followed by the rhizomes extract of *Zingiber officinale* with IC_{50}= 19. 1053 *and Phyllanthus urinaria* leaf (IC_{50} = 36. 57) while others exhibited the DPPH radical scavenging capacity ranging from 11-56% in ethanolic solvent.

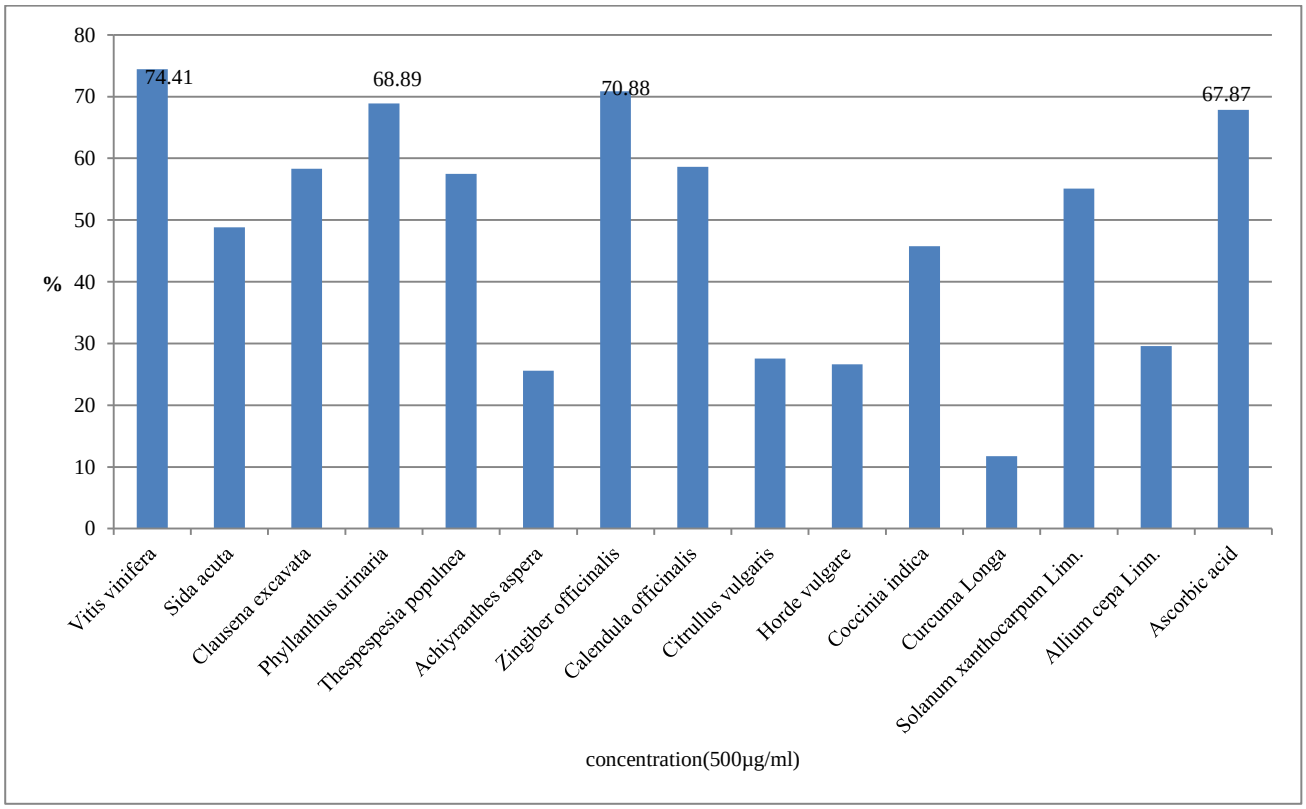

Figure1. Quantitative Determination of Antioxidant Activity *(in vitro* DPPH free radical Scavenging assay) by using 96 well plate reader

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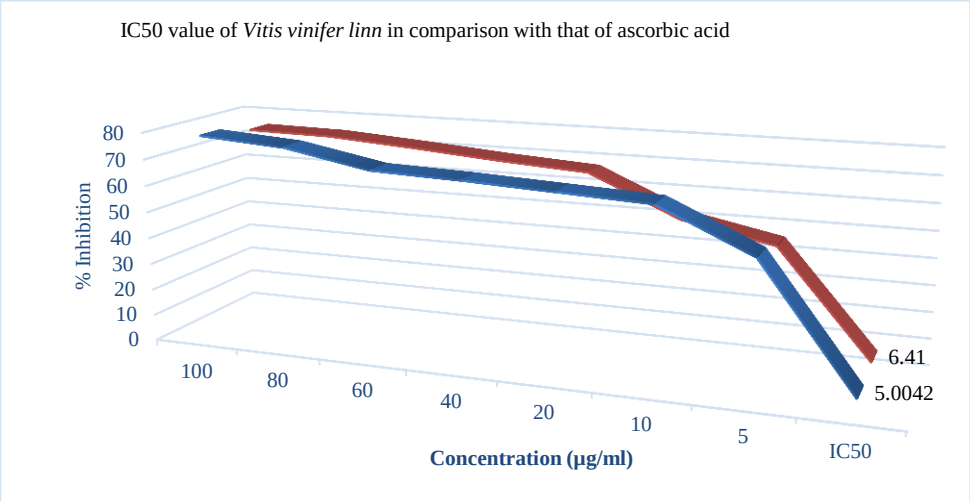

Figure 2(a)

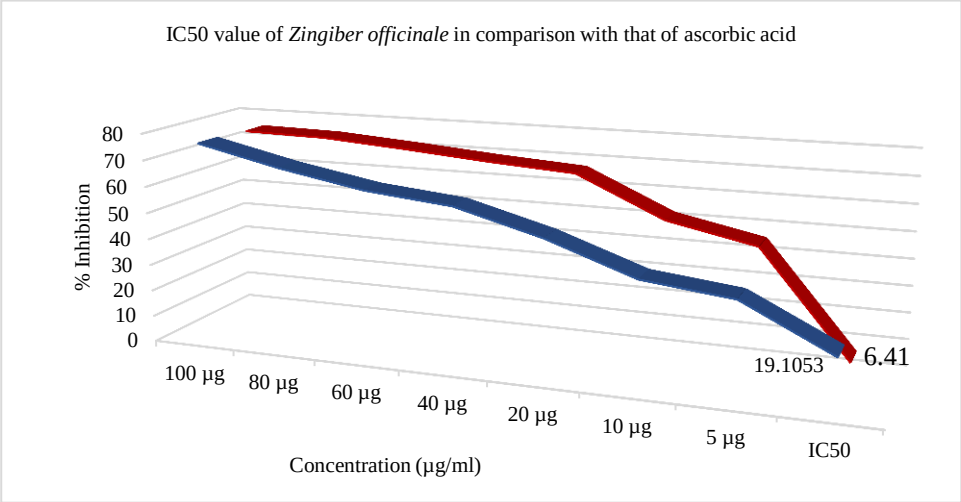

Figure 2(b)

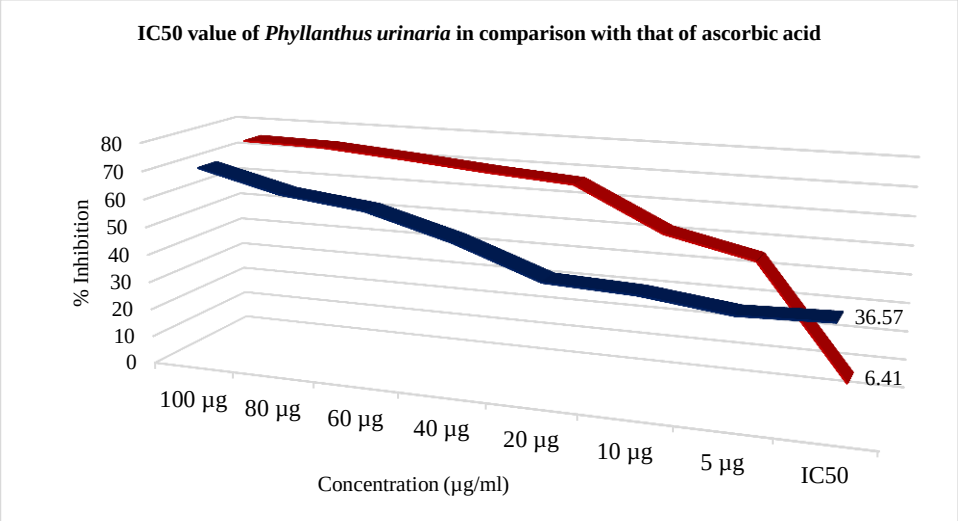

Figure 2(c)

Figure 2. IC_{50} values of (a) *Vitis vinifer*, (b) *Zingiber officinale* and (c) *Phyllanthus urinaria*. The blue line represents and shows the IC_{50} value of sample and the red one shows that of Ascorbic acid.

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D. Determination of total phenolic contents in the plant extracts:

There was a wide range of phenol concentrations in the medicinal plant infusions analyzed as shown in Figure 3. The values varied from 9 to 327mg of GA/g of extract as measured by Folin-Ciocalteau method. It is well known that plant polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations [15]. Between 14 medicinal plants, the highest phenol content was found in *Vitis vinifer Linn* seed. According to the Singleton and Rossi (1965) various phenolic compounds have different responses in this assay. [14] The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented ortho and para[16]. Since these structural features of phenolic compounds are reportedly also responsible for antioxidant activity, measurements of phenols may be related to their antioxidant properties. [17]

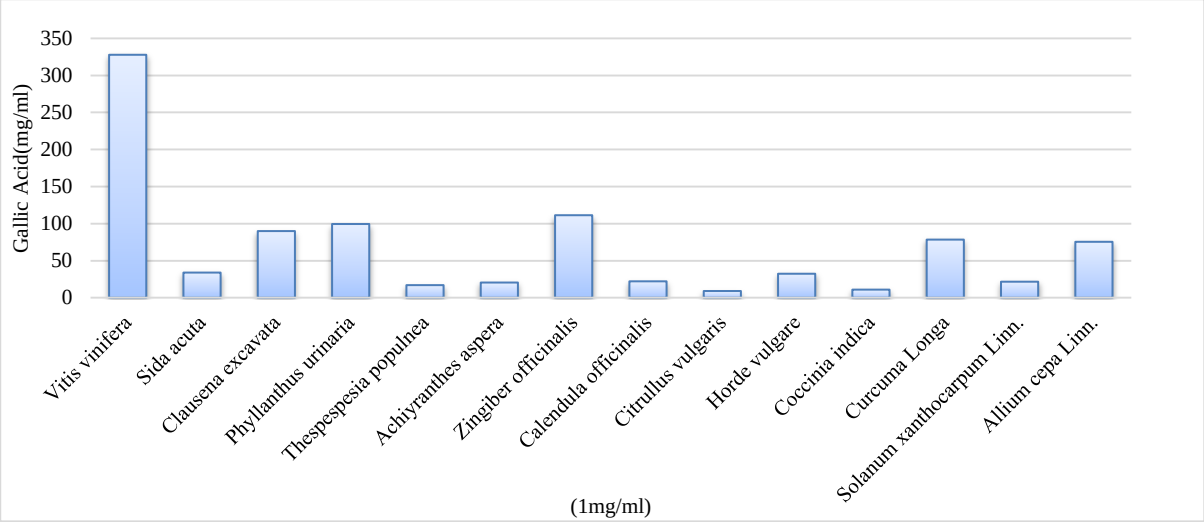

Figure 3. Total phenol content in different plant extracts.

E. Measurement of reducing power:

We investigated the reducing capacity of medicinal plants by measuring Fe3+-Fe2+ conversion. The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity [18].

For the FRAP test results, the highest value was obtained for *Vitis vinifera* (0. 96 ± 0. 022), followed by *Zingiber officinale* (0. 632 ± 0. 017) and *Phyllanthus urinaria* (0. 607 ± 0. 016) (Figure 4). The lowest result was given by *Achiyranthes aspera* (0. 42 ± 0. 001). The results showed that the reducing capacities for radical-scavenging of all the tested medicinal plants were even higher than that of the positive control(ascorbic acid).

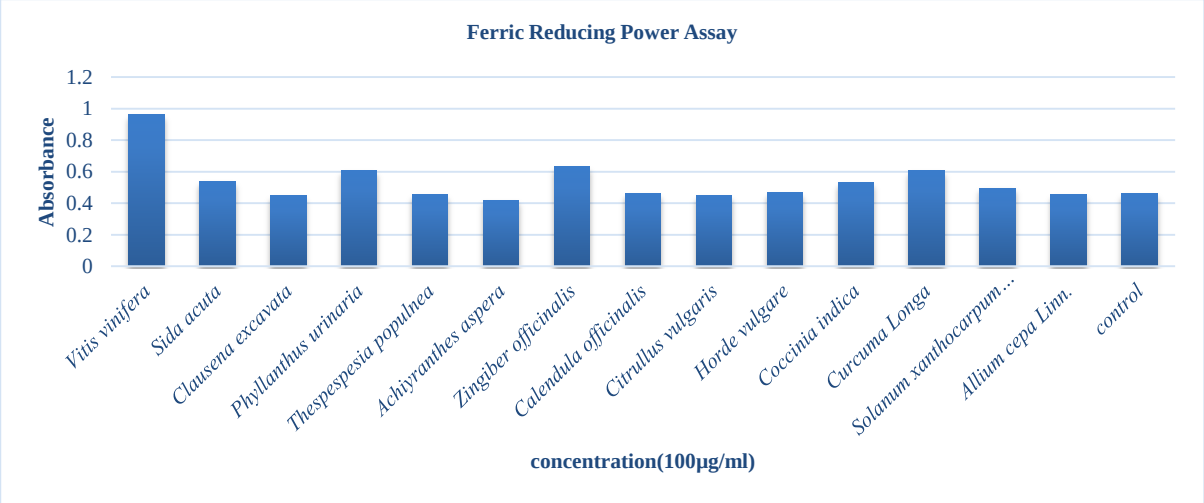

Figure 4. Ferric Reducing Power Assay of selected medicinal plants

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F. Detection of antimicrobial activity:

The development of microbial resistance to presently available antibiotics led the search for new antimicrobial agents [19]. Due to the problem of microbial resistance to antibiotics, attention is given toward biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antimicrobial activities [20]. Our search for antimicrobial bioactivity from tropical medicinal plants revealed antimicrobial activity of fourteen medicinal plants. Results of antimicrobial tests of the plant extracts are listed in Table 4.

Fourteen ethanolic extracts of the plant belonging to 11 families were tested against gram-positive and gram-negative pathogenic bacteria. Maximum antibacterial activity was shown by ethanol extract of *Sida acuta*, *Phyllanthus urinaria* , *Z. officinale* and *Calendula officinalis* against *Enterococcus faecalis* which can cause urinary tract infection(UTI) in human with the inhibition of 12-14 mm zone size. Ethanol extracts of *Vitis vinifer Linn*, *Sida acuta*, *Phyllanthus urinaria* , *Z. officinale* and *Calendula officinalis*. were found to be active with the inhibition of 11-14 mm against *Bacillus cereus*. None of the tested medicinal plants showed the activity against *E. coli*. The ethanol extract of *Vitis vinifer Linn*, *Sida acuta*, *Phyllanthus urinaria* , *Z. officinale Calendula officinalis Caccinia Indica* and *Curcuma Longa* show the antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (skin diseases causing bacteria).

In the anti-candidal assay , only ethanolic extract of *Sida acuta* showed potent activity to inhibit *C. albicans* growth. *C. albicans* is thought to be the major fungal pathogen of humans [21]. Furthermore, infections caused by *C. albicans* remain the predominant nosocomial fungal infections, due to the increasing population of patients whose immune systems are compromised by AIDS or immunosuppressant or anticancer therapy [22] and [23]. The ethanolic extract of *Clausena excavate*, *Achiyranthes aspera*, *Citrullus vulgaris*, *Horde Vulgare* , *Salanum xanthocarpum Linn* and *Allium cepa Linn* show no inhibition zones for all tested bacteria. . This probably explains the use of these plants by indigenous people against a number of infections since generations. The results here had shown that sven plants extracts are potentially rich in antimicrobial compounds.

Table 4 Antimicrobial activity of selected medicinal plantsextracts

Plant saamples	**Inhibition Zone(mm in diameter)**				
	Enterococcus faecalis	***Bacillus cereus***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Candida albicans***
Vitis vinifer Linn.	0	12±0. 39	12	11±0. 44	0
Sida acuta	14±0. 35	14±0. 23	11±0. 78	12±0. 66	14±0. 22
Clausena excavata	0	0	0	0	0
Phyllanthus urinaria	14±0. 67	0	0	0	0
Thespespesia populnea	10±0. 99	0	0	0	0
Achiyranthes aspera	0	0	0	0	0
Zingiber officinale	12±0. 45	11±0. 34	12±0. 56	11±0. 77	0
Calendula officinalis	12±0. 6	11±0. 33	11±0. 32	12±0. 55	0
Citrullus vulgaris	0	0	0	0	0
Horde Vulgare	0	0	0	0	0
Caccinia Indica	0	0	11±0. 45	11±0. 32	0
Curcuma Longa	0	0	12±0. 55	12±0. 33	0
Salanum xanthocarpum Linn.	0	0	0	0	0
Allium cepa Linn.	o	0	0	0	0
Control Ampicillin (10µg/25µl)	20±0. 23				

IV. CONCLUSIONS

The present work has proved that the tested extracts possessed strong antioxidant and antimicrobial properties. Based on these results, it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. Due to their antibacterial and antioxidant activities, *Vitis vinifer Linn*, *Zingiber officinale* and *Phyllanthus urinaria* extracts have promising potential as a source of natural antioxidant and antimicrobial agents. These results encourage the researchers to do further *in vitro* and *in vivo* research that will explore the role of bioactive constituents responsible for these activities. Hence, furtherstudies are needed at molecular level to evaluate the antioxidant activity and antimicrobial activity of their partially purified fractions.

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REFERENCES

[1] Ahmad, S. “Oxidative stress and antioxidantal defenses in biology”. New York: Chapman & Hall, 1995

[2] Davies, K. J. A. “Oxidative stress, antioxidant defenses and damage removal, repair and replacement systems” . IUBMB Life, 50, 279-289, 2000

[3] Tachakittirungrod, S.; Okonogi, S. ; Chowwanapoonpohn, S. “Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract”. Food Chem. 2007, 103, 381–388.

[4] Halliwell B and Gutteridge JMC . “Role of free-radicals and catalytic metal ions in human disease: an overview. Methods Enzymol”. 186: 1- 85, 1995

[5] Rimbach G, Fuchs J, Packer L. “Application of nutrigenomics tools to analyze the role of oxidants and antioxidants in gene expression” Raton Publishers, FL, USA, pp. 1-12, 2005.

[6] Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS and Heinonen M. “Antioxidant activity of plant extracts containing phenolic compounds. ” J. Agric. Food Chem. 47:3954-3962, 1999.

[7] Moure, A. ; Cruz, J. M. ; Franco, D. ; Dominguez, J. M. ; Sineiro, J. ; Dominguez, H. ; Nunez, M. J. ; Parajo, J. C. “Natural antioxidants from residual sources”. Food Chem. 2001, 72, 145–171.

[8] 8. Lai LS, Chou ST, eta l. , (2001). “Studies on the antioxidative activities of Hsiantsao *(Mesona procumbens. Hemsl.)* leaf gum. ” J. Agric. Food Chem. , 49: 963-968, 2001.

[9] Cao G, Sofic ER and PriorRL. “Antioxidant capacity of tea and common vegetables. J. Argic. Food Chem. ” (1996)44: 3426-3431.

[10] Irawan Wijaya Kusuma et. al “Antimicrobial and Antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia” Food Science and human wellness(2014), Volume 3

[11] Teng Wah Sam, “Bioactive natural products” pp442-456, CRC press, Inc. Penang(1993)

[12] Kumarasamy, Y. , Byres, M. , etal. ”Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother. Res21:615-621.

[13] Gow Chin Yen and Pin Der Duh, ”Scavenging effect of methanolic extracts of peanut hulls on free radical and active-oxygen species” Journal of Agricultural and food chemistry (1994)Volume 42, 629-632

[14] . Singleton, V. L. , & Rossi, J. A. "Colorimetry of total phenolics with phosphor-molybdic-phosphotungstic acid reagents" . American Journal of Enology and Viticulture, 16, 144-158. 1965

[15] Harborne, J. B. , "New naturally occurring plant polyphenols . In polyphenolic phenomena" Paris:Scalbert, INRA, 1993.

[16] Frankel, E. N. , Waterhouse, A. L. , & Teissedere, P. L. , "Principal phytochemicals in selected Califonia wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. "Journal of Agricultural and Food Chemistry, 43, 890-894. 199, 1998

[17] V. Katalinic and et-al, "Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols", Food chemistry 94(2006) 550-557

[18] Meir, S. , J. Kanner B. Akiri, and S. P. Hadas. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem. 43: 1813-1817. (1995)

[19] J. Parekh, N. Karathia, S. Chanda "Screening of some traditionally used medicinal plants for potential antibacterial activity" Indian J. Pharm. Sci. , 68 (2006), pp. 832–834

[20] Z. C. Maiyo, R. M. Ngure, J. C. Matasyo, et al. "Phytochemical constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species"Afr. J. Biotechnol. , 9 (2010), pp. 3178–3182

[21] J. Karkowska-Kuleta, M. Rapala-Kozik, A. Kozik

[22] "Fungi pathogenic to humans: molecular bases of virulence of *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus"*Acta Biochim. Pol. , 56 (2009), pp. 211–224

[23] G. J. Alangaden "Nosocomial fungal infections: epidemiology, infection control, and prevention Infect. Dis. Clin. North Am. , 25 (2011), pp. 201–225Article

[24] W. R. Jarvis "Epidemiology of nosocomial fungal infections, with emphasis on Candida species" Clin. Infect. Dis. , 20 (1995), pp. 1526–1530