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Study on Antioxidant and Antitumor Activities of Some Herbal Extracts

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***Abstract*—**The potential of antioxidant activities of the plant extract *Gynura procumbens, Achyranthes aspera* and *Polygenum tomentosum* were studied by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) .Antioxidant activity was qualitatively and quantitatively determined. In this analysis , Ascorbic acid (Vitamin C) was used as the standard .The antioxidant activities were observed all three plant extracts and the EC_{50} values of *G procumbens A.aspera* and *P.tomemtosum* were 13.7 µg /ml,14.37 µg /ml and 14.35 µg /ml. Among these plants, *G.procumbens* is more potent antioxidant activity then others. Antitumor activities were found with *A.aspera* (s2) extracts in the dose of 100ppm in carrot disks and *G.procumbens* (s1) and *P.tomentosum* (s3) in the dose of 1000 ppm. Therefore, these herbal plants are used in traditional medicines.

***Keywords*—**Antioxidant activity, Antitumor activity, DPPH, *G.procumbens.*

I. Introduction

SEVERAL plants are used in folk medicine to treat different illness of human beings. *Gynura Procumbens, Achyranthes aspera* and *Polygonum tomentosum* Willd are commonly used in traditional treatment of many aliments. In this study, a new approach was done to estimate accurately the potential capacities of the extracts of *G procumbens A.aspera* and *P.tomemtosum.G.procumbens* belongs to the family *Asteraceae* that has 1100 genera and 20,000species. *Gynura* consists of about 50 species of annual or perennial herb, subshurbs and sometimes scandent shrubs. *Gynura procumbens* (Merr.), which is known as "Sambung nyawa", is widely used in South East Asian countries in the traditional treatment of many ailments. It is also called bee's tail plant in Myanmar. It is an important medicinal plant indigenous to Malaysia, Indonesia, Thailand and Myanmar. *G.procumbens* has been used in hypertension, urinary infection, antidiabeties, anti inflammatery, and anti allergic agents. *G.procumbens* can reduce the cholesterol rate and decrease the high blood pressure, [1]. It is also used in medication for kidney failure, dysentery and throat infection. Besides that, it can be used to stop the bleeding, overcome menstrual cycle problems, and improve the kidney function, for animal bite[1, 2, 3].

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A.aspera belong to family Amarantaceae (A. L. de Jussieu, 1789 nom. conserv., the Spurge Family) consists of 160 genera and approximately 2400 species of shrubs, herbs, climbers. Different parts of the plant are ingredients in many native prescriptions in combination with more active remedies. In Western India the juice is applied to relieve toothache. The ashes with honey are given to relieve cough; the root is given at bedtime for night blindness, and rubbed into a paste with water it is used as an anjan (eye salve) in opacities of the cornea, [4].

Polygonum tomentosum Willd. is a perennial, rhizomatous floating creeper that grows in swamps and marshy areas in China, Taiwan, India, Indonesia, Malaysia, Burma, the Philippines, Sri Lanka, and Thailand. In Myanmar, a decoction of roots is used to mitigate stomachaches in children [5,6,7,8] .

The main objective of the research was to investigate the extracts which have large bioactive compounds for medical research.

II. Materials and Methods

A. Plant Material

Fresh leaves of *Gynura procumbens,Polygonum tomentosum* and roots of *Achyranthes aspera* were collected in December 2009 in Yangon, Mandalay and Magway Divisions, Myanmar. The collected samples were cleaned and air-dried at room temperature. Finally the samples were crushed and blended into powder form and stored for further analysis. Plant species (fig.1) was verified by an authorized botanist from Ministry of Science and Technology.

(a) (b) (c)

Fig. 1 (a)*Gynura procumbens,(b) Achyranthes aspera* and *(c) Polygonum tomentosum* plants

B. Preparation of Crude Plant Extracts

Air-dried powdered plant samples (1000g) were percolated with 95% ethanol. Maceration was carried out in ethanol solvent for one month at room temperature. The solvent of

extract material were filtered with Whatman No.1 filter paper. The filtrates were concentrated by rotary evaporation. The concentrated plant extracts were dried and evaporated on water bath. The extractive value of the plant samples were calculated in terms of percentage (%) in weight of the dried samples. The dried materials were stored at -4ºC.

C. Preliminary Phytochemical Analysis

Preliminary phytochemical examination of *G.procumbens* ,*P.tomentosum and A.aspera* were analyzed by qualitative method to screen the presence of some classes of compounds, starch, carbohydrate, tannin, saponin, α amino acid, acid or base or neutral, cyanogenetic glycoside, alkaloid, phenolic compound, flavonoids and steroids.[9,10]

D. Screening for for antioxidant activity

Antioxidant activity of ethanolic extracts of samples were determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitave assay.

E. Qualitative assay:

1) Dot-Blot DPPH Staining Procedure:

According to Soler Rivas et al. (2000), the dot-blot test is to compare radical scavenging capacity (RSC) of various products. This assay was used to establish whether different extracts of *G.procumbens* , *P.tomentosum* and *A.aspera* (ethyl acetate, ethanol, and methanol) had radical scavenging activity. All the extracts were dried and redissolved in ethanol. Aliquots of 5 µl (of a 10 mg/ml final concentration) of each extractant were applied on Merck Silica gel F254 plates and allowed to dry for a few minutes. A 0.4 mM DPPH solution in methanol was sprayed on the plates until they were evenly covered, [11, 12, 13].

2) 96-Multiwell Plate Assay

Aliquots of 0.5 ml of 0.04mM DPPH solution in methanol were applied into each well of 96-multiwell plate. Aliquots of 0.5ml (of a 50 mg/ml, 40mg/ml, 30mg/ml, 20mg/ml and 10mg/ml concentration) of each extract were then added immediately into each well except the control(only DPPH solution). In this assay ,the concentration of the extracts were dropped on the plate in the increasing order; control,10mg/ml,20mg/ml,30mg/ml,40mg/ml and 50mg/ml.The control was base row and the 50mg/mlwas at the top row. L-ascorbic acid (Vitamin C) was used as positive control for comparative study of plant extracts and black tea extract.

F. Quantitative antioxidant assay

1) Quantitative Determination of Antioxidant activity (In Vitro DPPH Free Radical Scavenging Assay)

For antioxidant (AOX) activity,the samples were determined spectrophotometrically using DPPH free radical scavenging assay. In this assay, 1 ml of various concentrations (5, 10, 15, 20 and 25 µg/ml) of ethanol extract of G.procumbens, A.asperaand P.tomentosum were mixed with 2 ml of 0.1mM DPPH solution in methanol. The mixture was allowed to react at room temperature in the dark for 30 minutes. Blank solutions were prepared with each test sample solution only when negative control was DPPH solution. L-ascorbic acid (Vitamin C) was the positive control. L-ascorbic acid (Vitamin C) was used as standard reference. Tea extract was used for comparing of antioxidant activity with the selected plant extracts. The decrease in absorbance was measured at 518nm using spectrophotometer.

Percentage antioxidant activity (AOXA%),effective concentration (EC_{50}) values, were calculated from The absorbance .The lower the EC_{50} values are the more effective antioxidant activity. The results are also expressed as the mg vitamin C equivalents/mg dry wt and are calculated as follows: Zero mg/ml was taken as 100%.For statistical analysis, the results were expressed as mean ± SEM and the EC_{50} values obtained from the linear regression plots .

G. *Preliminary screening for antitumor activity(Carrot Disc Bioassay)*

`Carrots(Daucas carota L.) were collected from local market in Mandalay, Myanmar. Carrot discs (7 mm x 5mm) were made with cork borer and carrot samples were sterilized with commercial bleach (Cocorex, Malaysia) followed by washing with DDW for three times.They were transferred to peridish. Each disc was overlaid with 50 µL of Agrobacterium tumefacien(10^8 cfu mL^{-1})except negative control.A fter inoculation,each carrot disc was added with 50 µL of two different concentrations(100ppm1nd1000ppm) extracts. These 5 discs were placed on Petri dish. Each dish was sealed by parafilm and incubated in laminar flow (control environment; 25-30°C). This test was carred out with all extracts as the same procedure. After 3 weeks, the discs were checked for young galls (tumors) developing from the meristematic tissue around the central vascular system.[14]

III. RESULTS AND DISSUSION

A. Preliminary Phytochemical Analysis

For identification of types of compound containing in the selected plants, preliminary phytochemical tests were performed. According to the results, all the test samples did not contain cyanogenic glycoside and presence of alkaloid, saponin glycoside, phenolic compounds, tannins and flavonoids.

B. Dot-Blot DPPH Staining Result

The results of dot-blot assay showed colored spots where the aliquots of different fractions of each extract and/or different extracts, black tea (C.sinesis) extract and L-ascorbic acid (vitamin-C) were dropped. The purple area on the plate indicates no free radical scavenging (antioxidant) activity and the yellow area indicates free radical scavenger or antioxidant activity. The more intense the yellow colour, the greater the antioxidant activity is as shown in Fig- 2 and Fig-3. The yellow colour can be masked by chlorophyll. These results indicate that all of the selected herbal plants (*G.procumbens,A.aspera and P.tomentosum)*have potential antioxidant activity. It was observed between the radical scavenging capacity and polarity of the extracts. The more polarable extracts give the more intense colour and the greater the antioxidant activity.

Fig.2 Scan of dot-blot test of a TLC Plate spotted with 0.4mM DPPH solution in methanol after fractions of each extract (n-hexane fraction, ethyl acetate fraction, ethanol fraction, methanol fraction) and L-ascorbic acid (vitamin-C) was applied. Control is 0.4mM DPPH solution in methanol.

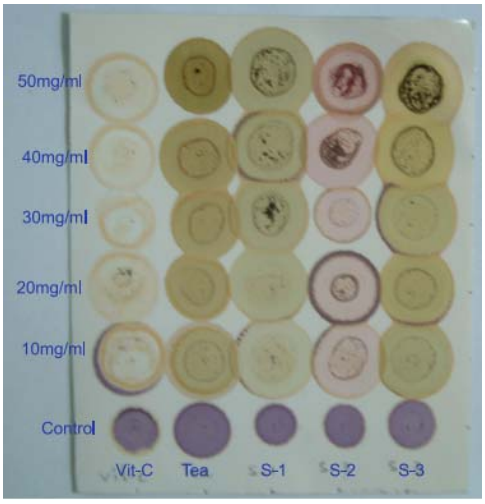

Fig.3 Scan of dot-blot test of a TLC Plate spotted with 0.4mM DPPH solution in methanol after extracts of each plant leaves (*G.procumbens,A.aspera and P.tomentosum)*), extract of Black Tea(*C.sinesis*) and L-ascorbic acid (vitamin-C) with various concentrations (10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, 50mg/ml) was applied. 0.4mM of DPPH in MeOH solution is control.

C.96-Multiwell Plate Assay

The different extract's colour reactions with DPPH were measured by a multi-well plate reader and the result are as shown in Fig.4. Colour formation with DPPH is indicative of antioxidants in excess (and that the concentration of the plant extracts is too high, like top row in right hand side of Fig-4 (vitamin-C) and pink of free radicals in excess (and that the concentration of the plant extracts is too low), like the first bottom row of right hand side of Fig-4 (vit-C). Threfore a concentration range is sought where the yellow colour just disappears or becomes translucent before pink appears. The colour reaction shows a gradual change from yellow to pink and indicates that optimum concentration range has been reached.

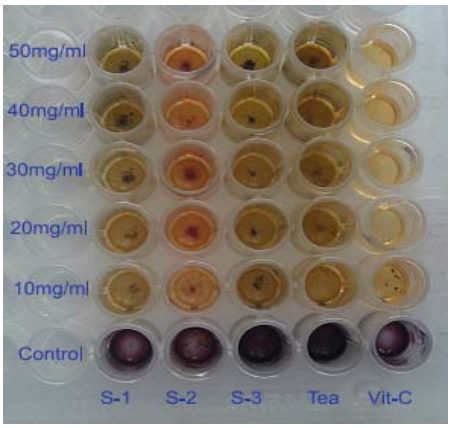

Fig.4. Part of 96 multi-well plate, showing the gradual colour change compared of each extract with that of black tea and vitamin-C at different concentrations after addition of 0.4mMDPPH solution in methanol

D.In vitro DPPH Free Radical Scavenging Assay

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic or crude extracts of plants. DPPH is a relatively stable radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent.

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from loses colour stochiometrically depending on the number of electrons taken up. After reduction, samples'color change from purple to yellow which can be quantified by its decrease of absorbance at wavelength 518 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability.

Free radical scavenging activity of the selected plant extracts , extract of black tea and the standard antioxidant Vitamin-C are shown in Table 1. The EC_{50} values (the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%) were calculated and are listed in this table. In the resuts from this table, EC_{50} value of *G.procumbens* extract show less than that of *A.asperaand P.tomentosum*) extracts. The results of free radical scavenging activity also showed *G.procumbens* has the strongest activity among the three plant extracts with 52.81% at 13.995ug/ml (its EC_{50} value) concentration. The extracts of

A.aspera and P.tomentosum were followedby G.procumbens. (50.59% at 50.57ug/ml (its EC_{50} value) concentration).

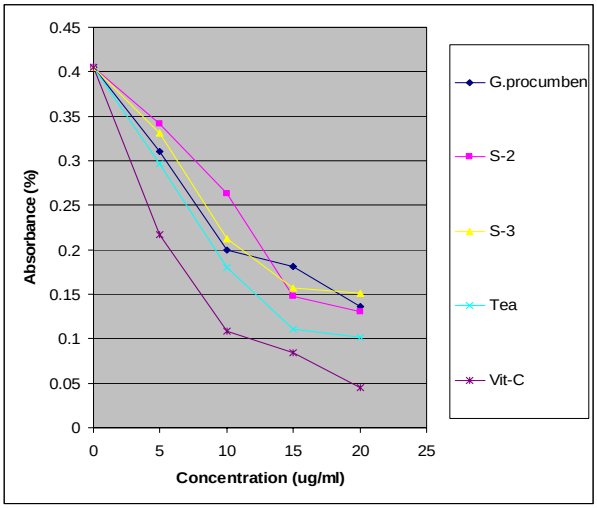

Fig. 5 Decrease in Activity of DPPH Free Radical in Reaction with Selected Plant Extracts at Various Concentration

More Scavenging capacities of vitamin C and black tea extracts have been found. None of the samples evaluated here showed activity as strong as the standard antioxidant Vitamin-C. Figure 5 illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the each plant extract , black tea and the standard ascorbic acid, as a reference compound, presented the highest activity at all concentrations. The EC_{50} values were found to be 13.7ug/ml,14.37 µg/ml and 14.35µg/ml for *G.procumbens, A.asperaand P.tomentosum* extract respectively. Scavenging activity is expressed as percentage of inhibition of DPPH free radical.

Fig.6. also shows that all selected plants have potential antioxidant activity like black tea and vitamin-C.

Antioxidant activity of plant extracts in mg Vitamin C equivalent has the benefits that the antioxidant activity is quantified and different plant extracts are comparable. Compared to 1mg of dry weight vitamin C equivalent of extracts are a little lower than that of Tea. *G.procumbens, A.aspera* and *P.tomentosum* show almost half and over half of the value of antioxidant activity of vitamin-C respectively. All selected plant extract here gave positive scavenging capacity (antioxidant activity) with DPPH.

TABLE 1
RADICAL SCAVENGING ACTIVITY OF SELECTED EXTRACTS AND STANDARD ANTIOXIDANTS ON DPPH FREE RADICAL

Sample	EC_{50} (ug/ml) mean ± SD	I% or Free Radical Scavenging Activity (%)	mg equivalent Vit-C/ mg dry weight extract
S- 1	13.7± 0.417	52.81	0.59
S-2	14.37 ± 0.106	50.59	0.58
S-3	14.35 ± 0.806	50.57	0.57
Tea (*C.sinesis*)	11.70 ± 0.367	53.61	0.71
Vitamin-C	8.31 ± 0.327	61.49	1

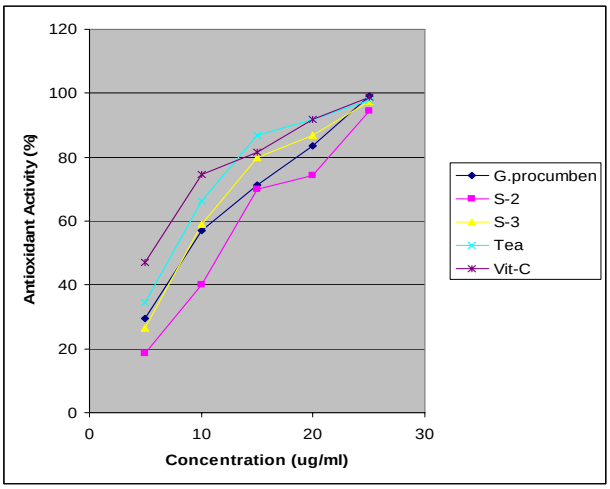

Fig. 6 Antioxidant Activity(AOXA%) of Selected Plant Extracts on DPPH Free Radical

E. Tumor Activity on Carrot-Disc Assay

The extracts showed activities in the cytotoxic assay are subjected to carrot disc assay to confirm the antitumor potential of medicinal plants (13). This assay can be routinely employed as a comparatively rapid, inexpensive, safe and statistically reliable prescreen for antitumor activity. In the present study, two different concentrations (100 ppm and 1000ppm) of the three selected extracts were done and there was a significant difference in tumor induction by the three strains and also tumor inhibition by the two concentrations of extract (Table 2). No gall was detected in carrot discs treated with *A.aspera* (s2) extracts in the dose of 100ppm and in carrot disks treated with *G.procumbens* (s1)and *P.tomentosum* (s3)in the dose of 1000 ppm and the test results are shown in Fig.7, Fig.8 and Fig.9.

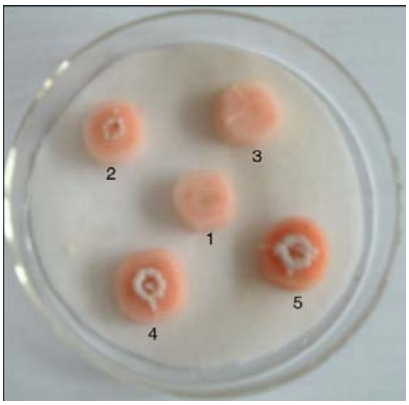

Fig. 7 Anti-tumor activity of **s-1** on carrot-disk assay with *A.tumefaciens*
(1) without treatment
(2) *A.tumefaciens* + **s-1** extract (100 ppm)
(3) *A.tumefaciens*+ **s-1** extract (1000 ppm)
(4) (+)ve control (*A.tumefaciens* + 70%EtOH)
(5) (-)ve control (*A.tumefaciens*)

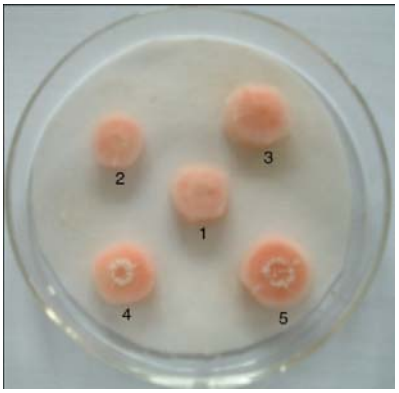

Fig. 8 Anti-tumor activity of **s-2** on carrot-disk assay with *A.tumefaciens*
(1) without treatment
(2) *A.tumefaciens* + **s-2** extract (100 ppm)
(3) *A.tumefaciens*+ **s-2** extract (1000 ppm)
(4) (+)ve control (*A.tumefaciens* + 70%EtOH)
(5) (-)ve control (*A.tumefaciens*

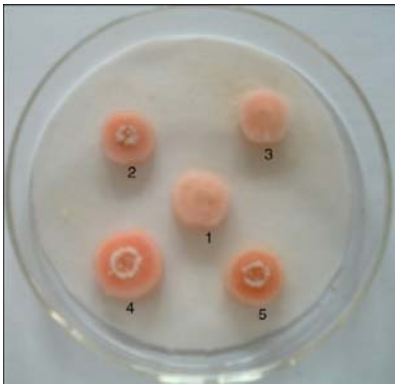

Fig. 9 Anti-tumor activity of **s-3** on carrot-disk assay with *A.tumefaciens*
(1) without treatment
(2) *A.tumefaciens* + **s-3** extract (100 ppm)
(3) *A.tumefaciens*+ **s-3** extract (1000 ppm)
(4) (+)ve control (*A.tumefaciens* + 70%EtOH)
(5) (-)ve control (*A.tumefaciens)*

IV. Conclusion

According to traditional knowledge, the selected plant samples have medicinal potentials. In the present study, information was confirmed at laboratory level by performing different biological assays. Significant antioxidant and antitumor activity was found, which varied in different species, but all values were in the acceptable range mentioned for specific assay by different authors. Further work is required to isolate and characterize the individual bioactive and to identify active compounds in these plant extracts.

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