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Quantitative Determination of Free Radical Scavenging Activity and Anti-tumor Activity of Some Myanmar Herbal Plants

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***Abstract*—**Antioxidant activities of ethanolic extracts of *Ardisia japonica* Blume., *Ageartum conyzoides* Linn., and *Cocculus hirsutus* Linn Diels. leaves was determined qualitatively and quantitatively in this research. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical solution was used to investigate free radical scavenging activity of these leaves extracts. Ascorbic acid (Vitamin C) was used as the standard. In the present investigation, it is found that all of these extracts have remarkable antioxidant activities. The EC_{50} values of these ethanolic extracts were 12.72 µg/ml for *A. japonica*, 15.19 µg/ml for *A. conyzoides*, 10.68 µg/ml for *C. hirsutus* respectively. Among these Myanmar medicinal plants, *C. hirsutus* showed higher antioxidant activities as well as free radical scavenging activity than black tea (*Camellia sinensis*), the famous antioxidant, and *A. japonica* and *A. conyzoides* showed a rather lower antioxidant activity than tea extracts. According to results from bioassay with carrot discs infected with *Agrobacterium tumefaciens*, all extracts showed anti-tumor activity after 3 weeks of incubation. No gall was detected in carrot disks treated with *C. hirsutus* and *A. japonica* extracts in the dose of 100ppm and in carrot discs treated with *A. conyzoides* extract in the dose of 1000 ppm. Therefore, the research clearly indicates that these weedy plants of dry farm land are exceptionally advantageous for human health.

***Keywords*—**Antioxidant, Anti-tumor activity, *C*arrot-disc bioassay, DPPH

I. Introduction

ALTHOUGH oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. The free radicals are very reactive oxygen species ($O_2^{\circ-}$, OH°, RO°, ROO°, H_2O_2, O_2*) produced as the result of an imbalance in the pro-oxidant/antioxidant homeostasis in the organism during the chemical reactions that contribute to the development and the maintenance of the cellular life. Free radicals toward endogenous molecules (DNA, proteins, lipids) having a beneficial role (antimicrobial activity) but implied especially in the pathology physiology of numerous affections: atherosclerosis, heart failure, liver injury, ageing, chronic inflammation, neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and ischaemic and haemorrhagic stroke), cancer, diabetes mellitus, and a plethora of other diseases. Under normal conditions, the body is equipped with defense mechanisms that scavenge reactive oxygen species (ROS) and protect the cell from oxidative damage.

However, the detoxifying enzyme processes get overwhelmed, saturated and faulty under conditions of low dietary antioxidant intake, inflammation, aging or exposure to environmental factors such as irradiation or tobacco smoke, including some enzymes like cyclooxygenase-2 (COX-2), lipoxygenase (LOX) and inducible nitric acid synthase (iNOS) that generate intermediaries that damage cellular macromolecules including DNA. The damage is made on proteins, lipids and nucleic acids signaling cascades leading to disruption of ion homeostasis and modification of the genetic apparatus, with consequence of apoptotic cell death. The brain is particularly very sensitive to oxidation stress possibly because of its high lipid content, high aerobic metabolic activity and low catalase activity.

Antioxidants (AOX) are considered a promising therapeutic approach as they may be playing neuroprotective (preventing apoptosis) and neurodegenerative roles. The main characteristic of an antioxidant is its ability to trap free radicals. In nature, AOX are grouped as exogenous or endogenous. The endogenous group includes enzymes (and trace elements part-of) like superoxidase dismutase (Zn, Mn and Cu), glutathione peroxide (Se) and catalase, and proteins like albumin, transferrin, ceruloplasmin, metallothionein and haptoglobin. The most important exogenous AOX are dietary phytochemicals (such as polyphenols, quinones, flavonoids, catechins, coumarins, terpenoids) and the smaller molecules like ascorbic acid (Vitamin C), alpha- tocopherol (Vitamin-E) and beta-carotene Vitamin-E, and supplements. The antioxidant processes occur in cytosol, mitochondria or in plasma.

Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free-radical-induced tissue injury. Many plant extracts and phytochemicals have shown to have free radical scavenging properties [1, 2] but generally there is still a demand to find more information

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concerning the antioxidant potential of plant species.

Belonging to the family Myrsinaceae, the *Ardisia japonica* Blume (Fig.1(a)) is a species of *Ardisia* native to eastern Asia, in eastern China, Japan and Korea. It is a low-growing evergreen shrub and plentiful in Middle Myanmar especially in Magway and Mandalay Regions [2]. Although species of *Ardisia* are a rich source of novel and biologically potent phytochemical compounds, such as bergenin and ardisin, the utilization of *Ardisia* species or their phytochemical constituents have not been fully explored, resulting in underexploitation of their uses.

Ageratum conyzoides L (Fig.1(b)) belongs to the family Asteraceae and is also ever green shrub and plentiful in Middle Myanmar. Traditionally it has been used in treatment of gastric motor activity, dysenteric, ulcer, maintenance of mucosal barrier integrity and skin diseases. *Cocculus hirsutus* (L) Diels (Fig.1(c)) belongs to the family Menispermaceae is every green climber shrub and widely growing throughout Myanmar [2]. It has sedative, hppotensive, bradycardiac, cardiotonic, spasmolytic and slight anticonvulsant actions in folklore medicine for the last few centuries. Traditionally, it is believed all these three kinds of plants have a stronger role in inhibiting the growth of *Mycobacterium tuberculosis*. The ongoing research is to study anti-tuberculosis activities of these selected Myanmar herbal plants [1, 3].

The results of preliminary phytochemical and chemical analysis reveal the presence of phenolic compounds and flavonoids in leaves of these plant, in this study described hereafter, the comparative antioxidant activity potential of the plant extracts were assessed against L-ascorbic acid (standard antioxidant) and tea extract (famous herbal antioxidant) using DPPH (1,1-diphenyl-2-picrylhydrazyl). Moreover, anti-tumor activities of plant extracts were also determined using *Agrobacterium tumefaciens* on carrot-disc assay. The information presented here also illustrates the potential of the genus as a source of therapeutic agents [6, 9]. Herbal folk medicines provide an interesting and still largely unexplored source for drug development with potential chemotherapeutic benefits.

(a) (b) (c)
Fig. 1 (a) Ardisia japonica (b) Ageratum conyzoides (c) Cocculus hirsutus

II. Materials and Methods

A. Collection, storage and preparation of plant materials

Leaves of *A. japonica*, *A. conyzoides* and *C. hirsutus* were collected and dried in the shade at ambient temperature, and ground to powder before extraction. A known mass of each sample was then soaked in ethanol for 1 month. The extracts obtained were concentrated under vacuum at 60°C using a rotary evaporator to give the crude extracts of each plant. The dry extracts were stored in sealed vials in the refrigerator prior to further processes [2].

B. Preliminary Phytochemical Analysis

Preliminary phytochemical examination of *A. japonica, A. conyzoides and C. hirsutus* were analyzed by qualitative method to screen the presence of some classes of compounds, glycoside, tannin, saponin, ∞ amino acid, acid or base or neutral, cyanogenic glycoside, alkaloid, phenolic compound and flavonoids [2].

C. Determination of Ash and Mineral Content

Determination of ash and mineral contents of *A. japonica, A. conyzoides and C. hirsutus* were done in Analysis Department, Research Centre, Ela, Ministry of Science and Technology [2].

D. Qualitative Determination of Antioxidant activity

1) Dot-Blot DPPH Staining Procedure

Because of antioxidant compounds are frequently highly polar compounds, two polar: ethanol, methanol, as well as an extractant of intermediate polarity: ethyl acetate and non-polar: n-hexane was selected. For the DPPH antioxidant assays and the Dot-Blot DPPH staining procedures, a final concentration of 10 mg/ml of each extractant was prepared by redissolving the dried extract in acetone. The prepared extracts were stored in tightly sealed glass containers at 5ºC.

Aliquots of 5 µl (of a 50 mg/ml final concentration) of each extractant were applied on Merck Silica gel F_{254} plates and allowed to dry for a few minutes. Drops of each sample were placed in a row. The sequence was according to increasing quantity: control (bottom row), 10mg/ml (1st middle row), 20mg/ml (2nd middle row), 30mg/ml (3rd middle row), 40mg/ml (4th middle row) and 50mg/ml (top row). The control was applied with 0.4mM DPPH solution in methanol only. A 0.4 mM DPPH solution in methanol was spotted on the each drop until they were evenly covered. L-ascorbic acid (Vitamin C) was used as positive control for comparative study of plant extracts and tea extract [5, 7, 10].

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 well which was used as control. The sequence was also according to increasing quantity. The plates were allowed to dry for a few minutes. Drops of each sample were placed in a row. The sequence was according to increasing quantity: control (bottom row), 10mg/ml (1st middle row), 20mg/ml (2nd middle row), 30mg/ml (3rd middle row), 40mg/ml (4th middle row) and 50mg/ml (top row). L-ascorbic acid (Vitamin C) was used as

positive control for comparative study of plant extracts and black tea extract.

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

Quantification of antioxidant (AOX) activity was determined spectrophotometrically using DPPH free radical scavenging assay [4]. In this assay, 1 ml of varying concentrations (5, 10, 15, 20 and 25 ug/ml) of ethanol extract of *A. japonica*, *A. conyzoides* and *C. hirsutus* 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 and/or has been used as standard reference. Tea extract was used to comparative study of antioxidant activity with the selected plant extracts. The decrease in absorbance was measured at 518nm using spectrophotometer. Values obtained were converted to percentage antioxidant activity (AOXA%) using the formula:

$$\text{AOXA \%} = 100 - \left\{\frac{A_{sample} - A_{blank}}{A_{control}} \times 100\right\}$$

Where, A_{sample} is the absorbance of the sample, A_{blank} is the absorbance of the blank and $A_{control}$ is the absorbance of the control.

Inhibition of free radical DPPH in percent (I%) (or) the DPPH free radical scavenging activity(%) was calculated from the absorption according to the following equation:

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

The antioxidant activity is expressed as effective concentration (EC_{50}) values. The lower the EC_{50} value, the more effective antioxidant activity is. The EC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of the test extracts (ug/ml) against the mean percentage of the antioxidant activity obtained from three replicate assays.

The results are also expressed as the mg Vitamin C equivalents/mg dry wt and are calculated as follows:

$$\frac{EC_{50}\ \text{Vit-C (mg/ml)}}{EC_{50}\ \text{sample (mg/ml)}} = \frac{\text{X.mg Vit-C equivalents}}{\text{mg dry wt}}$$

Zero mg/ml was taken as 100%.

For statistical analysis, the results were expressed as mean ± SEM (standard error of mean) and the EC_{50} values obtained from the linear regression plots (SigmaPlot ^{R} 2001, SPSS) showed a good coefficient of determination, with most values being $r^2 \geq 0.910$.

F. Determination of Anti-tumor Activity (Carrot Disc Assay)

Test for anti-tumor activity was done using carrot disc bioassay with minor modification [20]. Selected plant extracts were prepared with 100 ppm and 1000ppm concentration. Carrot (*Daucas carota* L.) samples were sterilized with commercial bleach (cocorax) followed by washing with sterilized deionized water for three times. Each disc was overlaid with 100ul of *Agrobacterium tumefaciens* inoculums (10^8cfumL^{-1}). A 50ul aliquot of each extract with different concentration was then added using syringe into disc. Petri dishes were sealed by parafilm and incubated at 30˚C. After 3 weeks, the disks were checked for young galls (tumors) developing from the meristematic tissue around the central vascular system.

III. RESULTS AND DISCUSSION

A. Phytochemical and Mineral Analysis

The phytochemical analysis of selected plant extracts had showed the presence of glycosides, flavonoids and phenolic compounds but had show the absence of cyanogenic glycosides. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds [4, 5]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [12]. The presence of polyphenolic compound in the selected herbal plants prompted us to study the free radical scavenging activity. According to the results of mineral analysis, there is absence of lead and arsenic in the selected plants revealed that these plants are potentially safe for further activity test.

B. Dot-Blot DPPH Staining Test

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. sinensis)* 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 (*A .japonica*, *A. conyzoides* and *C. hirsutus*) have potential antioxidant activity. It is extremely important to point out that, a strong correlation was observed between the radical scavenging capacity and polarity of the extracts. The more the polarity, the more the intense colour and the greater the antioxidant activity is.

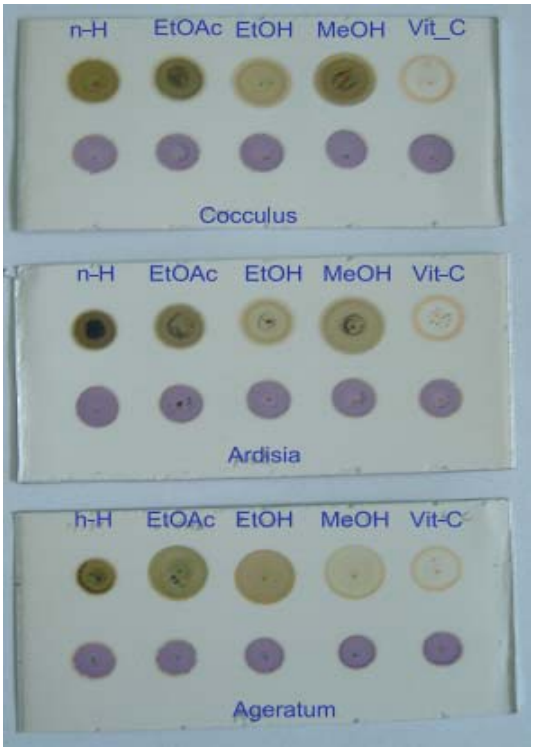

Fig.2 Scan of dot-blot test of a TLC Plate spotted with 0.4mM DPPH solution in methanol after fractions of each extract (n-hexane fraction, ethyl acetate fraction, ethanol fraction, methanol fraction) and L-ascorbic acid (vitamin-C) was applied. Control is 0.4mM DPPH solution in methanol.

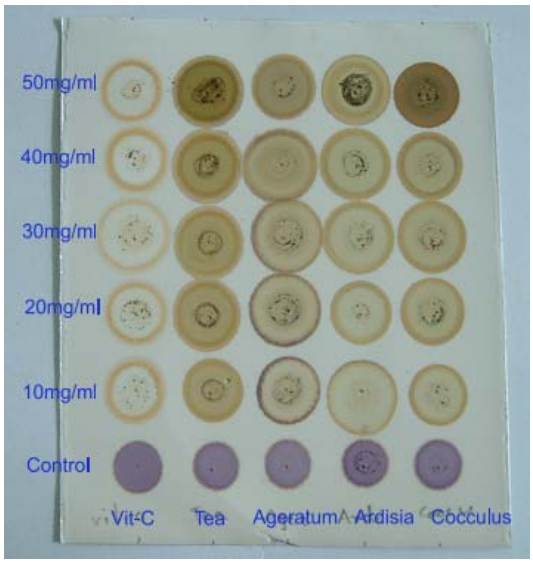

Fig.3 Scan of dot-blot test of a TLC Plate spotted with 0.4mM DPPH solution in methanol after extracts of each plant leaves (*A.japonica*, *A.conyzoides*, *C.hirsutus*), extract of Black Tea (*C.sinensis*) and L-ascorbic acid (vitamin-C) with various concentrations (10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, 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 the optimum concentration range has been reached.

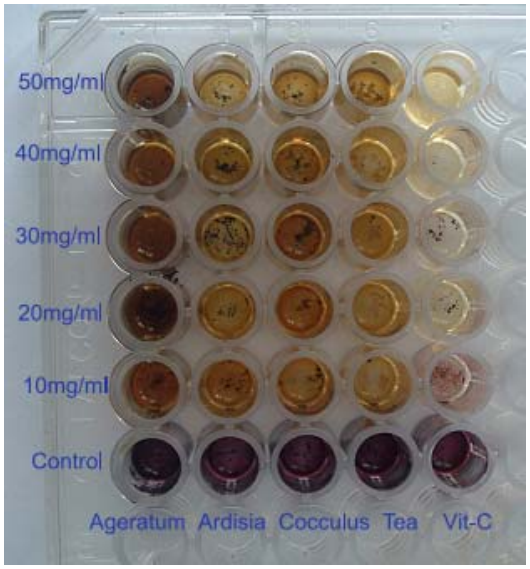

Fig. 4 Part of 96 multi-well plate, showing the gradual colour change compared of each extract with that of black tea and vitamin-C at different concentrations after addition of 0.4mMDPPH solution in methanol.

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 [15].

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, 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. The electrons become paired off and solution

loses colour stochiometrically depending on the number of electrons taken up.

Free radical scavenging activity of the selected plant extracts and 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. As can be seen from this table, EC_{50} value of *C .hirsutus* extract shows less than that of *A. japonica, A. conyzoides* and black tea (*C. sinensis*) extracts. The results of free radical scavenging activity also showed *C. hirsutus* have the strongest activity among the three plant extracts with 55.06% at 10.68ug/ml (its EC_{50} value) concentration. This extract was followed by Vitamin C (61.49% at 8.31ug/ml (its EC_{50} value) concentration). Scavenging capacities of the *A. japonica* and black tea extracts have been found almost equal. 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 12.72ug/ml, 15.19µg/ml and 10.68µg/ml for *A. japonica, A. conyzoides*, and *C. hirsutus* extract respectively. Data are reported as mean ± SD, n = 3. Scavenging activity is expressed as percentage of inhibition of DPPH free radical. 50% and above inhibition DPPH radical is considered as significant for scavenging activity.

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 (%)	EC_{50}Value (mg equivalent Vit-C/ mg dry weight extract)
A .japonica	12.72± 0.02	53.84	0.65
A. conyzoides	15.19± 0.11	50.56	0.55
C. hirsutus	10.68± 0.81	55.06	0.77
Tea (*C. sinensis*)	11.70± 0.37	53.61	0.71
Vitamin-C	8.31± 0.33	61.49	1

Antioxidant activity of selected plant extracts compared with standard antioxidant, Vitamin C and other phyto-antioxidant, black tea extract was shown in Fig.6. This figure also shows that all selected plants have potential antioxidant activity like black tea and Vitamin C.

Expressing plant extract's antioxidant activity in mg Vitamin C equivalent has the benefits that the antioxidant activity is quantified and different plant extracts are comparable. Compared to black tea where 1mg of dry weight, had Vitamin C equivalent of 0.71mg is a little lower than that of *C. hirsutus,* 0.77mg. *A. conyzoides* and *A. japonica* shows 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.

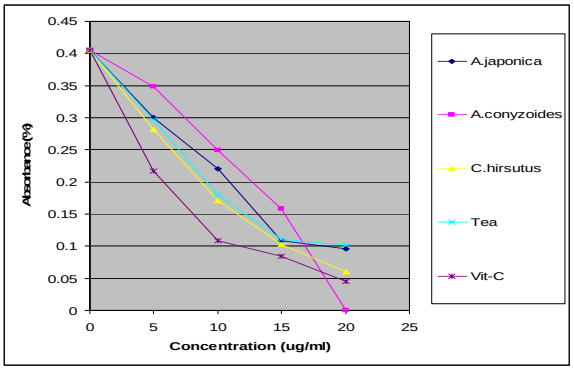

Fig.5 Decrease in Activity of DPPH Free Radical in Reaction with Selected Plant Extracts at Various Concentration

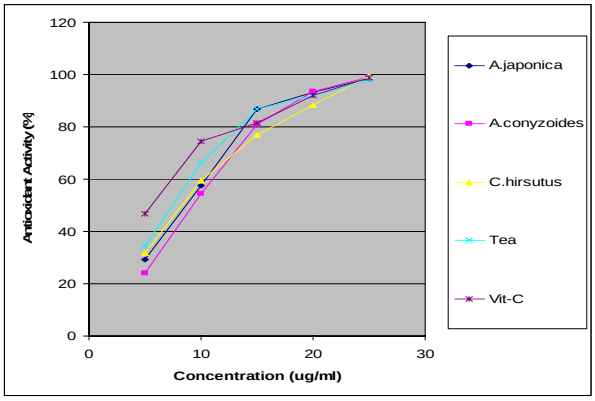

Fig. 6 Antioxidant Activity (AOXA%) of Selected Plant Extracts on DPPH Free Radical

E. Anti-tumor Activity on Carrot-Disc Assay

Test for anti-tumor activity was done using carrot disc overlaid with 100ul of *Agrobacterium tumefaciens* (10^8cfumL^{-1}). *A. tumefaciens* (*Rhizobium radiobacter*) is an indigenous soil bacterium known for its phytopathogenic effects. It causes crown gall tumor disease in a wide range of plants including most dicots, some monocots and some gymnosperms. Upon infection, the bacterium transfers part of its plasmid DNA to the plant. The Ti-plasmid causes the plant's cells to multiply rapidly without going through apoptosis, resulting in tumor formation similar in nucleic acid and histology to human and animal cancers [21]. It plays a vital role in aspect of antitumor studies. The T-DNA has also been transferred to human cells, demonstrating the diversity of insertion application. The mechanisms by which

Agrobacterium inserts materials into human cells also by type IV system, is very similar to mechanisms used by animal pathogens to insert materials (usually proteins) into human cells also type IV secretion. This makes *Agrobacterium* an important topic of medical research as well. Besides, it plays a vital rote in aspect of antitumor studies. After 3 weeks incubation of *A. tumefaciens* on each carrot disc in this research, negative control which use only for pathogenicity test showed young galls (tumors) developing from the meristematic tissue around the central vascular system. All extracts of selected plants showed anti-tumor activity. No gall was detected in carrot discs treated with *C. hirsutus* and *A. japonica* extracts in the dose of 100ppm and in carrot disks treated with *A. conyzoides* extract in the dose of 1000 ppm and the test results are shown in Fig.7, Fig.8 and Fig.9. 70% EtOH treated on the test disc was used in this case as positive control.

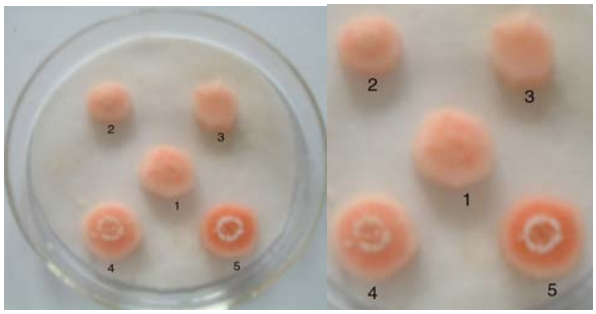

Fig. 7 Anti-tumor Activity of *A. japonica* on Carrot-disc Assay with *A.tumefaciens*
(1) without any treatment (2) *A. tumefaciens* + *A. japonica* extract (100 ppm)
(3) *A. tumefaciens*+ *A. japonica* extract (1000 ppm)
(4) (+)ve control (*A. tumefaciens* + 70%EtOH)
(5) (-)ve control (*A. tumefaciens*)

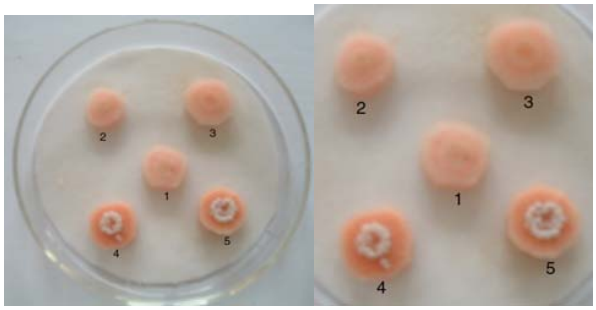

Fig. 8 Anti-tumor activity of *C. hirsutus* on Carrot-disc assay with *A. tumefaciens*
(1) without any treatment (2) *A. tumefaciens* + *C. hirsutus* extract (100 ppm)
(3) A. tumefaciens+ *C. hirsutus* extract (1000 ppm)
(4) (+)ve control (*A. tumefaciens* + 70%EtOH)
(5) (-)ve control (*A. tumefaciens*)

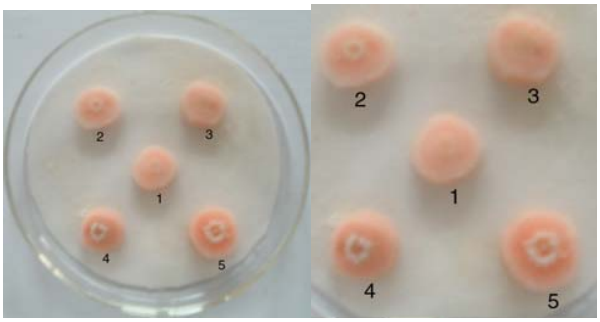

Fig. 9 Anti-tumor Activity of *A. conyzoides* on Carrot-disc assay with *A. tumefaciens*
(1) without any treatment (2) *A. tumefaciens* + *A. conyzoides* extract (100 ppm)
(3) *A. tumefaciens*+ *A. conyzoides* extract (1000 ppm)
(4) (+)ve control (*A. tumefaciens* + 70%EtOH)
(5) (-)ve control (*A. tumefaciens*)

IV. Conclusion

Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries, and these plants may offer a new source antioxidant activity.

According to the results from the phytochemical and mineral analyses, cyanogenic glycoside, lead and arsenic was not detected in *A. japonica, A. conyzoides and C. hirsutus.* So, we can assume that these plants are safe to use as medicinal plants.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic or crude mixtures of plants (methanol fraction of crude extract). In this investigation, DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH and the colour changes from purple to yellow after reduction. DPPH can be used to determine the proton radical scavenging action of extracts of the leaves of the selected plants, because it possesses phenolic compounds, a proton free radical. The more the polar capacity of the extract, the greater the antioxidant activity is.

The antioxidant activity of the different extracts differed. With these results, it should be take into account that the *in vitro* free radical scavenging potential of a substance or extract is related to its chemical properties in the medium tested and does not necessary reflect in vivo activity.

Tumor inhibiting ability of the extracts of selected plants finally confirmed them as all showed anti-tumor activity with its relevant concentrations.

From this study we can conclude that these three kinds of plants can be used as the source of typical diet or drugs of antioxidant activity and anti-tumor activity as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources

and belong to various classes of compounds with a wide variety of physical and chemical properties.

Purification of the bioactive component(s) from the extracts is underway and further investigations may improve our understanding of anti-cancerous potential.

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