Evaluation of Traditional Herbal Based Myanmar Medicine, Mansay

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Abstract- ***Traditional Myanmar medicine (TMM) has been practiced for thousands of years and at the present time is widely accepted as an alternative treatment for tumor and cancer. In this research work, one of the Myanmar traditional herbal based medicines, used as anti-tumor medicine, hot type medicine was chosen to study its Pharmacological activity. Myanmar name is Mansay. The phytochemical screening reveals the presence of several groups of secondary metabolites in this herbal based TMM. Free radical scavenging activity was assessed by DPPH assays and the antimicrobial activities were evaluated by disc diffusion method. Finally, a method utilizing brine shrimp (Artemia salina Leach) lethality was used to determine the toxicity of ethanol crude extract of Mansay.***

Indexed Terms- ***Herbal based Myanmar medicine, antioxidant, antimicrobial, cytotoxicity***

I. INTRODUCTION

Traditional or folk medicine comprises practices, approaches, knowledge and beliefs not based on scientific evidence that are applied to treat, diagnose and prevent illness within a society [1]. Ayurveda, the traditional Indian medicine (TIM) and traditional Chinese medicine (TCM) remain the most ancient traditions. These are the two 'great traditions' with experiential and experimental basis [2]. Humans have used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat diseases. Fossil researchers point out the human use of plants as medicines may be traced back at least 60,000 years [3, 4].

Natural products are important for the development of new drugs, and these products have been in constant use. Some type of medicines, such as anticancer, antihypertensive, and antimigraine medication, have benefited greatly from natural products [5, 6].

Natural products have a wide range of diversity of multidimensional chemical structures. They have been successfully employed in the discovery of new drugs and have exerted a far reaching impact on chemicobiology [7-9]. Artemisinin and its analogs developed from natural products are presently in wide use for the anti-malaria treatment which express how research using natural products has made a significant contribution in drug development [10, 11].

Among anticancer drugs approved in the time frame of about 1940–2002, approximately 54% were derived natural products or drugs inspired from knowledge related to such.

Different societies historically developed various useful healing methods to combat a variety of health- and life-threatening diseases. Traditional medicine is also variously known as complementary and alternative, or ethnic medicine, and it still plays a key role in many developed and developing countries today [12,13]. In traditional medicine there is usually a formal consultation with a practitioner or healer and such practices may be integrated into a country's heath care system, while in folk medicine advice is passed on more informally by a family member or friend and there is no such integration.

Traditional medicinal systems are challenging because their theories and practices strike many conventionally trained physicians and researchers as incomprehensible. The aim of this research is to determine the radical scavenging activities of one of the Myanmar's traditional herbal based medicine used as anti-tumor drug called Mansay and determine the

physical properties and cyto-toxicity of this herbal based medicine.

II. EXPERIMENTAL ANALYSIS

A. Sampling

The antitumor herbal medicine (Mansay) one of the Herbal based Myanmar Traditional medicines were purchased from Aung Thara Phu, Myanmar's Traditional Medicine shop, Zaycho Market, Mandalay.

B. Phytochemical screening and Mineral Analysis

Phytochemical tests were examined according to the standard methods as described by Harbone (1973) [14]. Moisture analysis was performed according to American Association of Cereal Chemists (AACC) method 44-15A [15]. The powdered sample (1 g) was measured into weighing crucible and placed in finance for 3 h. The samples were dried at 100±5ºC to constant weight. After cooling, the samples were weighed, and the moisture contents were calculated.

The ash content was determined using AACC method 08–01 [16]. The sample powder was measured into ash dishes in amounts of (3 g). Then samples were placed in a muffle furnace at 550 ± 10 ºC. They were incinerated until light gray ash, constant weight was obtained (7 h). After cooling, the samples were weighed, and the ash contents were calculated. Total ash was taken for the analysis of mineral contents. Bench-top Energy Dispersive X-ray Fluorescence (EDXRF) of make Rigaku elemental analyser with element range Na to U having Pd anode X ray Tube with high performance SDD detector with the use of NEX CG software.

C. Cytotoxicity Determination

In Cyto-toxicity bioassay, firstly the brine shrimp eggs were hatched. Three tea spoons brine shrimp eggs were placed in conical shaped vessel (one liter) through a vial containing sterile artificial seawater. It was controlled for under aeration 48 hours. Active nauplii used for this assay. After hatching twenty nauplii were drawn through glass capillary tubes and then placed in a vial containing 4.5 ml of brine solution and 0.5 ml of sample solution. It was maintained at room temperature for 24 hours under light. Beyond 48 hours, surviving larvae were counted. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} value was obtained from the best-fit line plotted concentration verses percentage lethality. Potassium dichromate was used as a positive control in the bioassay.

D. Determination of Antimicrobial Activities

The test organisms are gram positive species such as *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633) as and gram-negative species such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (ATCC 33459) which grown in Mueller Hinton agar at 37°C for 24 hours. Fungal species was unicellular *Candida albicans* (ATCC 60192) which grown in potato dextrose agar at ambient temperature for 72 hours. The antimicrobial test on these isolated bacterial were determined by disc diffusion assay [17]. EtOH extract was firstly prepared. Antimicrobial activities of EtOH extracts of Mansay were determined by the agar well diffusion method [18]. EtOH extract was dissolved in 80% ethanol to a final concentration of 10 mg/ml, and filter sterilized through 0.45-µm membrane filter. Small wells (6 mm in diameter) were made in the agar plates by sterile cork borer. 100 □l of the extracts was loaded into the different wells. In order to determine the antimicrobial efficacy of the fractions, aliquot of test culture (100µl) was evenly spread over the surface of the solidified agar. Ciprofloxacin (5µg/well) for bacteria, fluconazole (5µg/well) for fungi were used as positive control and EtOH was used as negative control for test microorganisms. All the pre-loaded plates with respective extract and test organism were incubated at 37°C, for 24 hours for bacteria and at 27°C. 48 hours for fungi. After incubation period, zone of inhibition was measured in mm (millimetre). All the tests were carried out in triplicate and their means recorded.

E. Determination of radical scavenging activity

The antioxidant activity of the extracts on the stable radical 1, 1-diphenyl;-2-picrylhydrazyl (DPPH) was determined by the method developed by Feresinet al. (2002) [19]. In this experiment, 1-1 diphenyl -2- picryl hradrazyl (DPPH) powder (stable free radical), Ascorbic acid (standard antioxidant) and Analar grade Ethanol (solvent) were used. The absorbance was

determined at 517 nm wavelength. The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometer. Firstly, DPPH solution, standard (Ascorbic acid) solution and sample solution were prepared. 10mg/ml concentration of four different extracts was prepared. These extracts were named as ethanol and watery extracted with cold and hot method (EC), (EH), (WC) and (WH), respectively.

1) Preparation of DPPH Solution: 2.346 mg of DPPH powder was dissolved in 100 ml of ethanol. This solution was thoroughly mixed at room temperature and it was stored in brown colored flask. This solution kept for no longer than 24 hours.
2) Preparation of Standard Solution: 1 mg of ascorbic acid was dissolved in 10 ml of ethanol. This solution was thoroughly mixed at room temperature to obtain 100 µg / ml of standard solution. The five concentrations such as 50, 25, 12.5, 6.25, 3.125 and 0.625µg/ml were prepared by using two fold dilution methods.
3) Preparation of Test Sample Solution: 0.002 g of sample was dissolved in 10 ml ethanol. This solution was thoroughly mixed at room temperature for 15 minutes to obtain 200 µg/ ml of sample solution. The concentrations of sample solutions (50, 25, 12.5, 6.25, 3.125 µg/ml) were also prepared by using two folds dilution method.

After preparation, 1 ml of each ascorbic acid solutions and 3 ml of DPPH solutions were thoroughly mixed and incubated for 15 minutes in the dark room at room. The absorbance of the mixture was measured at 517 nm. In the same way, 1 ml of each sample solutions and 3 ml of DPPH solutions were thoroughly mixed and incubated for 15 minutes in the dark room. The absorbance of the mixture was read at 517 nm.

$$\%\,\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is the calculation of percent inhibition value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. Ascorbic acid was used as a positive control in the bioassay. The IC_{50} (concentration providing 50% inhibition) was calculated graphically using a calibration curve vs. percentage of inhibition.

III. RESULTS AND DISUCSSION

Qualitative phytochemical analyzes of herbal medicine (Mansay) contain all the tested phytochemical constitutes including Saponin, Sterioid, Phenolic, Reducing Sugar, Alkaloid, Flavonoid, Glycoside, Polyphenol, and Tannin except terpene.

The moisture content of Mansay can change with the weather. All Myanmar traditional herbal based medicine can absorb the moisture from its environment. So the herbal based medicines should be stored carefully to maintain the quality.

Mansay contains lower ashes value (3.00%). EDXRF analysis point out Mansay contains Ca (9.048%), Cl (8.528 %), Si (7.027 %), K (2.945%), Fe (0.660%), Al (0.579%), P (0.403 %), S (0.322%), Ti(0.03%) and Mn(0.0311%), respectively. The EDXRF result reveals Mansay does not contain toxic heavy metal.

Cyto-toxicity of this medicine, Ethanolic extract of Mansay was determined at Department of Biotechnology, Mandalay Technological University, Ministry of Science and Technology. In this experiment, Potassium dichromate was used as positive control. LC_{50} values of less than 1mg/ml are considered as significantly active which suggests that crude extract has toxic upon cell. LC_{50} value for ethanolic extract of the Mansay was found to be 111.17±0.1884 ppm (0.111 mg/ml at 24 hour). This 0.111 mg/ml concentration is over dosage limit for patient. Because the traditional physician permissible dosage of this drug on patient is 150 mg, its ethanolic extract was found to be 0.0825 mg/ml. The patient should be avoided to take over dosage.

Free radical scavenging activity of four extract EH, EC, WH and WC and the standard Ascorbic acid were determined. The IC_{50} value of extracts are found to be WC > WH > EC > ascorbic acid >EH. It means that WC extract has highest antioxidant activity. The IC_{50} values are 49.00, 61.74, 14.73, 11.23 and 11.99 µg/ml for EC, EH, WC, WH and Ascorbic acid, respectively. It means watery cold extract captured more free

radicals formed by DPPH resulting into decrease in IC_{50} value.

CONCLUSION

Myanmar traditional medicine has flourished over thousands of years and has become a distinct entity. DPPH free radical scavenging assay was studied for evaluation of antioxidant potential and antioxidant potential of this medicine can express in µl with vitamin C equivalent which has the benefits that the antioxidant activity was quantified. According to DPPH assay, watery cold extract has higher antioxidant activity than standard ascorbic acid. Based on the collective results, Mansay, Myanmar traditional herbal based medicine exhibits signifying that this medicine is also a few toxic to humans when taken over dosage. Nevertheless, as toxic compound expressions can be insignificant in vivo, further investigation of this compound by using in vitro method should be pursued.

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ACKNOWLEDGMENT

The authors would like to express their profound gratitude to the Department of Higher Education, Ministry of Education, Naypyitaw, Myanmar for provision of opportunity to do this research. We would like to express our profound gratitude to all members of Editorial Board of IRE, 2000 for their giving permission to participate in ICONIC RESEARCH AND ENGINEERING JOURNALS. We also thanks to Dr Kyaw Thar Htun, Pro rector of Technology University (Kyaing Tong) for his permission to write my research paper in this Journal.