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Evaluation of Total Phenolic Content and Antimicrobial Activity of the Bark of *Taxus baccata* Linn (Yew Tree)

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Contents

ယူးပင်အခေါက်၏ Phenolic ဒြပ်ပေါင်းများပါဝင်မှုနှင့် အဏုဇီဝပိုးမွှားများ ဟန့်တားနိုင်ဂုဏ်သတ္တိ ကို ဖော်ထုတ်ခြင်း

သီတာချို၊ သုတေသနလက်ထောက်-၂
လွင်လွင်အောင်၊ သုတေသနလက်ထောက်-၂

စာတမ်းအကျဉ်း

ဤသုတေသနပြုချက်တွင် ယူးပင်အခေါက်၏အနူဇီဝပိုးမွှားများ ဟန့်တားနိုင်ဂုဏ်သတ္တိ (Antimicrobial activity) ကိုဖော်ထုတ်ခြင်းနှင့် ယူးခေါက်တွင်ပါဝင်သော ဒြပ်ပေါင်းများ ပဏာမ စမ်းသပ်လေ့လာခြင်းများကို standard methods များအတိုင်း ဆောင်ရွက်ထားပါသည်။ ဖျော်ရည်(၄) မျိုးသုံး၍ ရရှိလာသော ယူးခေါက်ထုတ်နုတ်ပစ္စည်းကြမ်းများ(crude extract) ၏ အနူဇီဝပိုး (၆) မျိုး ဖြစ်သည့် *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Candida albicans* နှင့် *E-coli များအား* ဟန့်တားနိုင်ဂုဏ်သတ္တိ agar well diffusion method ကို သုံး၍ ဖော်ထုတ်ထားပါသည်။ Ethyl acetate (EtOAc) နှင့် Ethanol (EtOH) ယူးခေါက် extract များသည် အနူဇီဝပိုး (၆) မျိုးလုံးကို ဟန့်တားနိုင်စွမ်းပကား အလွန် များကြောင်း (high activity) တွေ့ရှိရပါသည်။ ယူးပင်အခေါက်တွင် alkaloid၊ flavonoid၊ glycoside၊ phenolic compound၊ polyphenol၊ lipophilic၊ saponin၊ steroid၊ reducing sugar၊ terpene and tannin phytochemical အသီးသီးပါဝင်နေကြောင်း စမ်းသပ်တွေ့ရှိပါသည်။ ဖော်ပြပါ phytochemical များအနက်မှ ethanolic extractive များတွင်ပါဝင် phenol၊ flavonoid၊ polyphenol နှင့် tannin phytochemical များသည် antioxidant action ရှိပါသည်။ ဤသုတေသနပြုချက်တွင် ယူးခေါက်ထုတ်နုတ်ပစ္စည်းပျော်ရည် (extract solution) ၏ total phenols content ကို စမ်းသပ်ဖော်ထုတ်ပါသည်။ Acid Property စမ်းသပ်ချက်, $FeCl_3$ အရောင်ပြ စမ်းသပ် ချက် နှင့် Ellagic Acid စမ်းသပ်ချက် ရလဒ်များမှလည်း phenolic compound များ ပါဝင် နေကြောင်း တွေ့ရှိရပါသည်။ ထို့အပြင် ယူးပင်အခေါက်တွင် total phenolic ဒြပ်ပေါင်းများ ပါဝင်မှုကို Folin-Ciocalteau ဓာတ်စမ်းပစ္စည်းကို အသုံးပြု၍ UV spectrophotometer (wavelength 765 nm) ဖြင့် တိုင်းတာခဲ့ပါသည်။ gallic acid ကို standard solution .အဖြစ် အသုံးပြုထားပါသည်။ phenolic ဒြပ်ပေါင်းများသည် antioxidant (ဓာတ်တိုးဆန့် ကျင်ပစ္စည်း) များ ဖြစ်ပါသည်။ antioxidant များသည် ခန္ဓာကိုယ်တွင်းရှိ free radical များဓာတ်တိုးမှုကြောင့် ရောဂါအမျိုးမျိုး ဖြစ်စေခြင်းကို ကာကွယ်နိုင်စွမ်းရှိပါသည်။ ဤသုတေသနပြုချက်အရ ယူးပင်အခေါက်၏ total phenolics ပါဝင်မှု ပဏာမမှာ ၄၃.၂၁ mg gallic acid equivalent (GAE) per 100 g ဖြစ်ကြောင်း တွေ့ရှိရပါသည်။

Evaluation of total phenolic content and antimicrobial activity of the Bark of *Taxus baccata* Linn (Yew Tree)

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Abstract

The present research work was designed to determine the antimicrobial activity of the extracts of the bark of *Taxus baccata* Linn. (Yew tree) and to investigate the preliminary phytochemical screening which was performed with standard methods. The antimicrobial activities of crude extract of the bark of *Taxus baccata* L. on six types of organisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Candida albicans* and *E-coli*. were determined by agar well diffusion method with four solvent systems. The ethanol and ethyl acetate extracts gave high activity on all tested organisms. Phytochemical screening of the bark of this selected plant, Yew tree consists of alkaloid, flavonoid, glycoside, phenolic compound, polyphenol, lipophilic, saponin, steroid, reducing sugar terpene and tannin respectively. These phytochemical screening revealed the potent source of different phytochemical constituents on ethanolic extractive including, phenol, flavonoid, tannin that are responsible for antioxidant action. In this research work, the extract solutions of the bark of *Taxus baccata* L. were checked for qualitative test of total phenols. It responds positive for Acid Property test, Color with $FeCl_3$ test and Ellagic Acid test respectively. Moreover, The total phenolic content (TPC) of the barks of *Taxus baccata* L was measured by Folin-Ciocalteau assay using UV spectrophotometer (UV- 1800, SHIMADZU, UV spectrophotometer) at 765 nm and gallic acid was used as standard. This phenolic component is responsible for antioxidant activity. Phenolic compounds prevent free radicals oxidation which leads to various deseases. The total content of phenolics in the bark of *Taxus baccata* (L), determined spectrophotometrically using the Folin-Ciocalteu reagent , was 43.21 mg gallic acid equivalent (GAE) per 100 g dry weight.

Key words: Antimicrobial activity, Phytochemical screening Antioxidant activity, phenolic, Gallic acid

1. Introduction

Taxus baccata Linn., is an evergreen tree belongs to the family Taxaceae that includes three genera and nearly fifteen species. The most well known taxus species are *Taxus baccata* (English yew, Common yew, Himalayan yew, Bhutanese yew), *Taxus brevifolia* (Western yew, Pacific yew), *Taxus Canadensis* (American yew) and *Taxus cuspidate* (Japanese yew). Among them, *Taxus baccata* has a wide distribution in Asia, ranging across Afghanistan Pakistan, India, Nepal, Bhutan, China, Indonesia, Malaysia, Myanmar, Vietnam and the Philippines (Mulliken T, et.al., 2008). In Myanmar, it could be found in the Kachin State and Chin State especially in Namataung mountainous area. The genus *Taxus baccata* Linn. has interested many researchers since the discovery of the anti-cancer agent taxol, a diperpenoid alkaloid originally isolated from the bark of Picific yew, *Taxus brevifolia* (Satyajit Dutta* et.al.,2010). All plant parts of t*axus baccata* Linn. with the exception of the arillus, which is enveloping the seeds, contain toxic taxine alkaloids (Wilson et al., 2001). They have been implicated in many human and animal poisonings. Although due to the toxic taxane alkaloid content it has rarely been documented as a folk remedy. Taxol, isolated from the bark extract of yew trees, *Taxus brevifolia* Nutt. is an important drug for the treatment of different kind of cancers, as well as AIDS-related Kaposi's sarcoma (Brambilla L, et.al., 2008). Bark are reported to be used in traditional medicine as an analgesic, antimalarial, antirheumatic, emmenagoque, sedative, antispasmodic, aphrodisiac and anti-asthmatic (E. Kupeli, *et al*., ,2003).

Copious scientific studies have suggested that the chief source of compounds with free radical scavenging activity is phytochemical found in fruits, herbs, vegetables, and natural products (Rachh *et al*., 2009). Among the phytochemicals, phenolic constituents are well-recognized free radical scavengers. They have the ability to halt or prevent cell oxidation, scavenge free radicals, inhibit lipid peroxidation and other free radical-mediated processes (Ningappa *et al*., 2008).

Plants are one of the main sources of reliable bioactive compounds such as secondary metabolites and antioxidants. Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic (Milan S. Stanković, 2010). Phenolic compounds are secondary metabolites as well as antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Phenolics are regarded as the molecules with the highest potential to neutralize free radicals. Sahu, N. and Saxena, J. 2013 reported that these compounds act mainly as antioxidants due to their ability to scavenge free radicals and chelate metals *in vitro* and *in* vivo. Therefore, high consumption of phenolic compounds leads to reduce risk of cardiovascular diseases and cancers. Plant phenolics have been reported to have a lot of biological activities including anti-carcinogenic, antioxidant and anti-mutagenic (Biju, J., et al 2014). Natural antioxidants, derived from plants, are also secondary metabolites, mainly plant phenolics such as phenolic acids, flavonoids and carotenoids, which are amongst various antioxidants produced by plants for their sustenance (Apak R, et al.., 2007). Antioxidant activity of plants might be due to their phenolic compounds (Cook N.C. and Samman, S., 1996). Due to their natural origin, the

antioxidants obtained from plants are of greater benefit in comparison to synthetic ones (ROHMAN *et al*., 2010). The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect (CHEN *et al*., 1992). Therefore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous. The plant kingdom consists of an invaluable source of new chemical products, which may be important due to their biological properties and their potential use in medicine.

Myanmar has vast and variety of medicinal plants. They contain varying degrees of chemicals and they have a direct impact on physiological activity. Most of the people in Myanmar use the Yew tree for the treatment of diseases and to relief pain. The medicinal important of a plant is due to the presence of some special substances like phenolic compounds. So, the scientific studies of its bioactive chemical compounds and usage in therapy play a very important role. The basic aim of the research was to determine antimicrobial activity in various extracts of the bark of *taxus baccata* L, and the preliminary phytochemical screening as well as to examine total phenolic content using spectrophotometric methods. Moreover, this research has been made on the interesting upgrade level of Myanmar traditional medicine isolated from one of the NWFPs, the bark of t*axus baccata* L which have notable pharmacological effects and have been studied chemically.

2. The Objectives of the Present Research Work

The objectives of the present research work are as follows.

- To evaluate the content of total phenolic compounds in the bark of yew.
- To provide information on the effective chemical constituents of the bark of yew

3. Literature Review

3.1 Description

It has recently been proposed that the genus should be separated into 24 species with 55 varieties; neither approach has gained universal acceptance (Möller M,.et.al., 2013). The most common taxus species are *Taxus baccata* (English yew, Common yew), *Taxus brevifolia* (Western yew, Pacific yew), *Taxus Canadensis* (American yew) and *Taxus cuspidate* (Japanese yew) (Wilson *et al.*,2001). European yew grows in Europe, Canadian yew is distributed within North America and Himalayan yew is the species found in Asia(Parmar VS,et.al.,1999). Himalayan yew has a wide distribution in Asia, ranging across Afghanistan Pakistan, India, Nepal, Bhutan, China, Indonesia, Malaysia, Myanmar, Vietnam and the Philippines (Mulliken T, et.al., 2008). In historical documents from the Roman period, *Taxus baccata* (L) was used as an analgesic, antimalarial, antirheumatic, emmenagoque, sedative, antispasmodic, aphrodisiac and anti-asthmatic (E. Kupeli, *et al*., ,2003). *T. baccata* has been used in the Ayurvedic system for the treatment of cancer, diarrhea, asthma, haemoptysis and also used as carminative, expectorant, stomachic etc. (Yelne, M .B.,et al., 2005). *T. baccata* leaves are reported to be used in traditional medicine

as abortificient, antimalarial, antirheumatic and for bronchitis (Appendino, G., 1993). and dried leaves and barks are used against asthma (Sing, V., 1995). Anticancer (Jennewein, S. and R. Croteau, 2001). anti- inflammatory for the care of inflammatory and antinociceptive (Kupeli, et al., 2003), antifungal (Miros³awa, *et al*., 2003), antimycobacterial (Erdemoglu, et al.,, 2001) activity of *T. baccata* has been reported. The diterpenoid anticancer drug Taxol (generic name paclitaxel, ure 1) was first isolated from the bark extract of yew trees, *Taxus brevifolia* Nutt. Taxol is an important drug for the treatment of different kind of cancers, as well as AIDS-related Kaposi's sarcoma (Dhillon T, et al., & 2). The earlier researcher described that recovery of Taxol extracted from various parts of mature trees has been reported to be in the following order: bark > needles > roots > branches > seeds > wood. A large number of phytochemicals like taxoids viz. taxusin, lignans, flavanoids, steroids and sugar derivatives have been isolated from the various species of Taxus including *Taxus baccata (*Parmar VS,et.al., 1999). Lignans are known to possess various biological activities such as antibacterial, antifungal, antiviral, antioxidant, anticancer and anti-inflammatory effects (MacRae and Towers, 1984). Phenolic compounds prevent free radicals oxidations which lead to various diseases.

3 .2 Traditional uses and medicinal importance

For centuries, the stem bark of Himalayan yew has been used to make tea by the *Bhotiya* tribal community in the Garhwal region of Himalayas (Kala CP,2010), and to cure colds, coughs, hypertension and cancer in the buffer zone villages of Nanda Devi Biosphere Reserve (Maikhuri RK, et al 1998). Himalayan yew is used in Indian folk medicine for its aphrodisiac and sedative properties, as well as in the treatment of bronchitis, asthma, epilepsy, snakebites and scorpion stings (Mulliken T, Crofton P, 2008). Various preparations and parts of the plant have specific uses. Juice from the leaves is used to treat cancer and bronchitis; bark and leaf juice is used for asthma, bronchitis and cancer, whereas dried leaves are considered to be useful for asthma, bronchitis, hiccough, epilepsy, diarrhoea and headache (Kunwar RM, Shrestha KP, Bussmann RW 2010). A tincture made from the young shoots is used for treatment of feeble and falling pulse, coldness of extremities, headache, giddiness, diarrhoea and severe biliousness. Himalayan yew is also an important ingredient of several Ayurvedic formulations such as *lavanbhaskar churna*, *talisadivati*, and *sudarshan churna* (Chauhan NS. 1999):. A decoction prepared from the bark is used in the management of pain associated with muscles, joints and rheumatism whereas a decoction of the leaves is used for treating liver problems (Poudel RC et al.,2013). A decoction prepared from the bark, filtered and mixed with jaggary (a sweetener) is taken twice a day for 14 d in case of hysteria (Thakur S. 2011) and a decoction prepared from the stem is taken early in the morning to treat tuberculosis (Gangwar KK, et al., 2010). Some written works attribute antirheumatic, anticatarrhal, insecticidal and wound-healing properties to Himalayan yew and recommend the use of the drug in powder form for treatment of several disorders including vitiated blood, tumors, dermatosis and helminthiasis (Khare CP, 2007).

3.3 Phenolic Compounds

Phenols (phenolic) are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites, organic molecules that are not directly involved in normal growth, development and reproduction of the plant. Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic (Borneo *et al*., 2008).They are also known as phytochemical or natural products, which have significant medicinal value (Khin Maung Lwin, 2010). Polyphenol compounds are one of the important group of secondary metabolites and bioactive compounds of plants. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*), and partiallyresponsible for the overall organoleptic properties of plant foods (Jin Dai, et.al, 2010). The largest and best studied natural phenol are the flavonoid which include several compounds, among them flavonoid, flavones, flavan-3 ol, flavanone, anthocyanidin and isoflavonoid. Some flavonoids are valued pharmacologically for their antiinflammatory and antihepatotoxic properties. Many disorders in human organism such as atherosclerosis, arthritis, Alzheimer disease, cancer etc.**,** may be the result of increased concentrations of free radicals in an organism. Antioxidants are specific organic compounds that destroy single oxygen molecules, also called free radicals. Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. These free radicals are unstable molecules that can freely react with and destroy healthy cells (Hnin Kyawt Wai, 2015). Reactive oxygen species (ROS) and nitrogen (RNS) species, as the most frequent pro-oxidants, either originate from normal metabolism or are induced by UV radiation and different pollutants. Harmful effects of disturbed antioxidant-prooxidant balance can be largely prevented by intake of antioxidant substances (GHOSH *et al*., 2008).

3 .4 Classification of Phenolic Compounds

Phenolics are classified according to the structural complexity and biosynthetic origin.

The most important groups are

(i) Anthocyanins and anthochlors

(ii) Benzofurans

(iii) Chromones and chromenes

(iv) Coumarins

(v) Minor flavonoids

(vi) Flavones and Flavonols

(vii) Isoflavonoids and neoflavonoids

(viii) Lignans

(ix) Phenols and phenolic acids

(x) Phenolic ketones

(xi) Phenyl propanoids and (xii) Quinones

4. Materials and Methods

4.1 Sample Collection

The bark of *Taxus baccata* (L) was collected from Namataung, Chin State in the month of March. The plant was identified and authenticated by the Botanists from Wood Anatomy and Tree Improvement Sections.

4.2 Sample Preparation

Fresh bark was cleaned firstly. They were chopped into small pieces by using a knife and scissor and then allowed to air dry in the well ventilated room at temperature 40 ± 2°C for about three weeks. These air dried samples were used throughout the experiment.

4.3 Preliminary Phytochemical Screening of the Bark of *Taxus baccata* L.

The extract of the bark of *Taxus baccata* L. were subjected to preliminary phytochemical qualitative screening for the presence or absence various secondary metabolites following the standard procedures.

4.3.1 Test for Alkaloid

The dried sample (2g) was boiled with 1% hydrochloric acid (10ml) for about 10 minutes, allowed to cool and filtered. The filtrate was divided into two portions and tested with 5 drops of Dragendroff's reagent and 5 drops of Wagnar's reagent respectively. The orange precipitate and reddish brown precipitate indicate the presence of alkaloid.

4.4.2 Test for Flavonoid

0.5ml of alcoholic plant extract was placed in the test tube. 5-10 drops of dilute hydrochloric acid and small pieces of magnesium was added in a test tube. And then, a few drop of concentrated H_2SO_4 were added to the mixture and boiled for a few minutes, which

gave pink colour. The appearance of pink, reddish pink or brown color indicates the presence of flavonoid.

4.4.3 Test for Glycoside

A few drops of 10% lead acetate solution were added to a small amount of alcoholic extract of sample in 1ml of water in a test tube. A yellow or white color indicates the presence of glycoside.

4.4.4 Test for phenolic Compound

A few drops of 10% ferric chloride solution were added to a small quantity of aqueous extract of sample in 2 ml of distilled water in a test tube. A blue or greenish blue color or precipitate indicates the presence of phenol.

4.4.5 Test for Polyphenol

The ethanol extract of sample (1 ml) was treated with 8 drops of the mixture of 1% ferric chloride (1% $FeCl_3$) and 1% potassium ferricyanide (1% K_3 [Fe $(CN)_6$]). A greenish blue color indicates the presence of polyphenol.

4.4.6 Test for Saponin

About 1g of dried sample was boiled with distilled water and then cooled and filtered. 1 drop of $NaHCO_3$ was added to the filtrate and shaken vigorously for 10 seconds in a test tube and allowed to settle for 10 minutes. The formation of froth indicates the presence of saponin.

4.4.7 Test for Steroid

The ethanol extract of sample (1ml) was placed in the test tube and was heated with pet ether on a water bath for 10 minutes. Then 2 drops of acetic anhydride and conc: H_2SO_4 (2 drops) were added to this test tube. The colour of green was observed which shows the presence of sterol.

4.4.8 Test for Sugar (Carbohydrate)

5 ml of Benedict's solution was added to 0.5 ml of aqueous extract of sample in a test tube and then boiled for 5 minutes. On cooling down, orange red precipitates indicate the presence of sugar.

4.4.9 Test for Terpene

A mixture of few drops of concentrated sulphuric acid, 2 drops of acetic anhydride and 2.5 ml of chloroform was added into ethanol extract of sample in a test tube. Formation of red color which turns to brown red indicates the presence of terpene.

4.4.10 Test for Tannin

1 ml of ethanol extract of sample was tested with 10% $FeCl_3$. Bluish black colours was obtained and then disappear on addition dilute sulphuric acid solution followed by the formation of a pale brown precipitate, it indicates the presence of tannin.

4.3.11 Test for Lipophenol

1 ml of sample extract was tested with 0.5 N KOH and 4 drops of NaOH was added. The deep colour solution (more than reagent colour) was obtained. Therefore, lipophenol may be present in this sample.

4.4 Antimicrobial Activities on Crude Extract of Sample

4.4.1 Sample Preparation

Four portions of the samples were placed in the four small bottles. Each of them were percolated with ethyl acetate, n-hexane, acetone and ethanol, respectively and sent to DCPT (Development Center for Pharmaceutical Technology), Insein, Yangon to determine the anti-microbial activities on six organisms, *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumalis*, *Pseudomonas aeruginosa*, *Candida albicans* and *E-Coli*.

4.4.2 Determination of Antimicrobial Activities

The antimicrobial activities of crude extract of sample on six types of organisms were determined by agar well diffusion method with four solvent systems. In this case, the nutrient agar medium was prepared and the study of antimicrobial activity was carried out as follow.

Firstly, nutrient agar was boiled and 20-25 ml of it was poured into the test tube and plugged with cotton wool and sterilized at 121°C for 15 minutes in an autoclave. After autoclaving, the tubes were cooled down to 30-35 °C and the agar in the tube was poured into sterilized petridish and 0.1-0.2 ml of test organisms were also added into the dish. These were allowed to set the agar for 2-3 hours. After the agar was set, 7 mm petals agar well were made by the help of sterilized agar well cutter. After that, about 0.2 ml of sample was introduced into the agar well and incubated at 37 °C for 24-28 hours. The inhibition zone appeared around the agar well indicates the presence of antimicrobial activity.

4.4.3 Qualitative Test for phenol

4.4.3.1 Extraction Procedure

300 g of dried plant sample was ground in a mortar and pestle. It was extracted with 500 ml of 0.3 % HCl in methanol. The mixture was centrifuge at 5000 rpm for 30 minutes. The supernatant was decanted to a small beaker. The extraction procedure was repeated two times. The supernatant was poured to the same container. It was evaporated to dryness and 200 ml of distilled water was added to dissolve the residue. This extract contains the phenols.

4.4.4 Qualitative Test for Phenols

4.4.4.1 Acid Property Test

The extract solution of the bark of *Taxus baccata* L. was tested with blue litmus paper.

When the paper was dipped in the solution, it turns red color which represents the content of phenolic compounds.

4.4.4.2 Color with $FeCl_3$

1ml of extract solution was taken and a few drops of very dilute solution of ferric chloride were added. The color changes to brown which indicates the presence of phenolic compounds.

$$6C_6H_5OH + FeCl_3 \longrightarrow 3H^+ + [Fe(OC_6H_5)_6]^{3-} + 3HCl$$

(Phenol) (Brown)

4.4.4.3 Ellagic Acid Test

The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or nigger brown precipitate occurred in the extract indicated the presence of phenols.

Figure (4.1) The results of qualitative tests for the the bark of *Taxus baccata* (L)

4.4. 5 Quantitative Determination of Total Phenolic Content

4.4.5.1 Preparation and Determination of Standard Ethyl Gallate

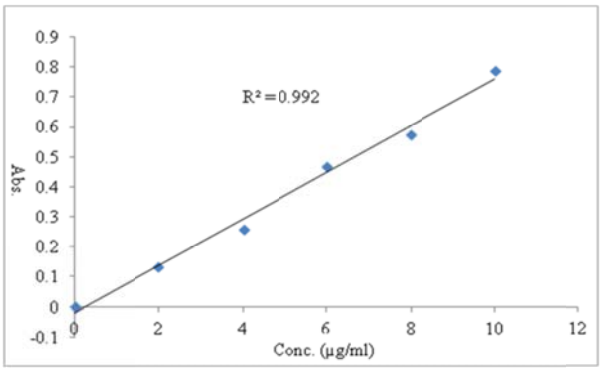
10 mg of the standard ethyl gallate was taken in a test tube. 100 ml of distilled water was added to the standard compound. 1 ml of this standard solution was taken in another test tube. The volume of this solution was made up to 10 ml with distilled water. The solution was taken by micro-pipette into a series of test tubes 20 µl, 40 µl, 60 µl, 80 µl, 100 µl respectively. The volume was made up to 1.6 ml with distilled water in each test tube. Then 100 µl of Folin-ciocalteau reagent and 300 µl of standard Na_2CO_3 (20%) solution were added. After each solution was heated in the water bath at 40 °C for 30 minutes, the absorbances of the solutions were measured with a UV spectrophotometer at 765 nm with respect to the blank solution. The calibration curve of standard ethyl gallate is shown in figure (4.1).

Figure (4.2) Prepared standard gallic acid solutions with different concentrations.

Table (4.1) The Results of Absorbances of Standard Ethyl Gallate Solution

No	Test Sample	Concentration (µg/ml)	Absorbance
1.	Std GA 1	2	0.134
2.	Std GA 2	4	0.258
3.	Std GA 3	6	0.467
4.	Std GA 4	8	0.575
5.	Std GA 5	10	0.805

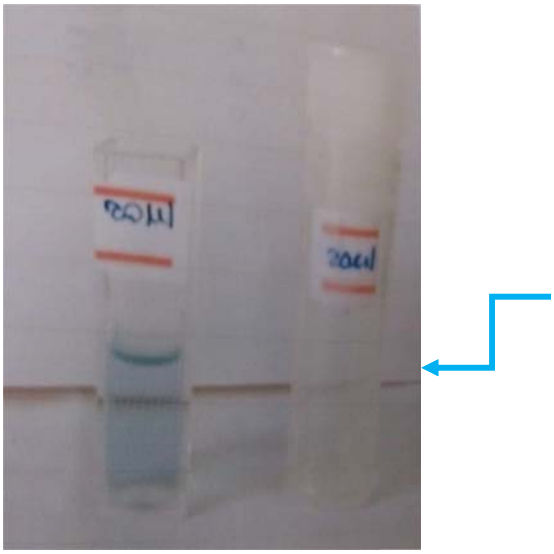
Standard Curve



4.4.5.2 Determination of Total Phenolic Content of the Bark of *Taxus baccata* L.

The total phenolic content of the bark extract of *Taxus baccata* L. was measured with the Folin -Ciocalteau reagent. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Firstly, 2 µl of extract solution was taken in a test tube. It was made up to 1.6 ml with distilled water. 100 µl of Folin -Ciocalteau reagent was mixed, then 300 µl of standard Na_2CO_3 (20%) solution was added.

The mixture was heated in a water bath at 40 °C for 30 minutes and then cooled in an ice bath. The absorbance of this prepared sample solution was measured by UV spectrophotometer at 765 nm. The total phenolic content of the extract was expressed as ethyl gallate equivalent (EGE) mg / g dry weight.



extract
cata L.

Table (4.2) Results of Absorbances and Concentration of Extract Solution (Sample Table)

No	Test Sample	Concentration (µg/ml)	Absorbance
1.	Yew 1	4.325	0.262
2.	Yew 2	4.323	0.260

4.4.5.3 Calculation

The total phenolic content of the bark extract of *Taxus baccata* L. was calculated from the standard curve prepared from different concentrations of ethyl gallate in which the same spectrophotometric procedure was followed for the working standard.

By using the resulted data,

2 µl of diluted extract solution contain µg of phenolic compounds.

2 ml of diluted extract solution contain mg of phenolic compounds.

20 ml of diluted extract solution contain = ?

$$= \frac{2o\ ml \times 4.324\ mg}{2ml}$$

$$= 43.21\ mg$$

The total phenolic content of the bark extract of

Taxus baccata (L) = 43.21 mg (ethyl gallate equivalent) / g

5. Results and Discussion

5.1 Phytochemical Examination

5.1.1 Preliminary phytochemical test of the bark of Taxus baccata L.

Before *t*he total phenolic content of the bark extract of *taxus baccata* L.was investigated, the phytochemical screening of the extract was performed as a first step. Preliminary Phytochemical analysis for alkaloid, flavonoid, glycoside, phenolic compound,

polyphenol, lipophilic, saponin, steroid, reducing sugar terpene and tannin were carried out by standard methods and each of tests was quantitatively expressed as negative (-) and positive (+).

Table (5.1) Preliminary phytochemical Test of the bark of Taxus baccata (L)

No.	Test	Reagent used	Observation	Result
1.	Alkaloid	Dragendroff's reagent	Orange ppt	+
2.	Flavonoid	EtOH, Conc: HCl ,Mg ribbon	Pink color	+
3.	Glycoside	10% Lead acetate	White ppt	+
4.	Phenolic	10% $FeCl_3$	Bluish black color	+
5.	Polyphenol	1% $K_3Fe(CN)_6$, 1% $FeCl_3$	Greenish blue color	+
6.	Lipophilic	0.5 N KOH solution	Deep color solution	+
7.	Saponin	Boiling with water	Froth growing	+
8.	Sugar	Benedict's solution	Brick red ppt	+
9.	Steroid	Acetic anhydride , Conc.H_2SO_4	Blue color	+
10.	Terpene	$(CH_3CO)_2O$,$CHCl_3$, Conc.H_2SO_4	Reddish brown color	+
11.	Tannin	1% $FeCl_3$	Yellowish brown ppt	+

(+) sign indicates the presence of the constituent

(-) sign indicates the abscence of the constituent

According to these tests, the root the bark of *taxus baccata* L. consists of alkaloid, flavonoid, glycoside, phenol compound, polyphenol, lipophilic, saponin, steroid, reducing sugar terpene and tannin respectively. Among these phytochemicals, tannin, flavonoid, phenolic, polyphenol are group of phenolic compounds. They are antioxidants which may provide protection against a number of diseases and observed responsible for the free radical scavenging effects. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

5.2 Antimicrobial activities of crude extract of the bark of *taxus baccata* L.

Antimicrobial Activities of the crude extract of the bark of various solvent extracts of *taxus baccata* L. were determined by applying Agar-well diffusion method on six selected organisms. . These results are tabulated in table (5.2).

Table (5.2) Antimicrobial activities of the bark of *taxus baccata* L.

Sample	Solvent extracted	Inhibition zone					
		I	**II**	**III**	**IV**	**V**	**VI**
Yew Bark	n-hexane	–	–	–	–	–	–
	Acetone	–	–	–	–	–	17 mm (+++)
	EtOAc	20 mm (+++)	40 mm (+++)	30 mm (+++)	23 mm (+++)	23 mm (+++)	27 mm (+++)
	EtOH	20 mm (+++)	40 mm (+++)	30 mm (+++)	23 mm (+++)	23 mm (+++)	27 mm (+++)
Control	n-hexane	–	–	–	–	–	–
	Acetone	–	–	–	–	–	–
	EtOAc	–	–	–	–	–	–
	EtOH	–	–	–	–	–	–

Agar Well – 10 mm

10 mm ~ 14 mm (+) = low activity

15 mm ~ 19 mm (++) = medium activity

20 mm above (+++) = high activity

Organisms:

I = *Bacillus subtilis*

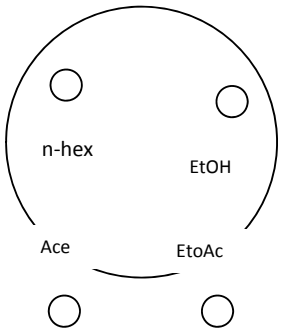
II = *Staphylococcus aureus*

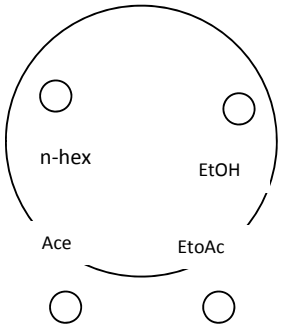
III = *Bacillus pumilus*

IV = *Pseudomonas aeruginosa*

V = *Candida albicans*

VI = *E.coli*





Sample **Control**

As the results of antimicrobial activities, ethanol and ethyl acetate extracts respond high activity on all six organisms, such as *Bacillus subtilis, Staphylococcus aureus Bacillus pumilus, Pseudomonas aeruginos, Candida albicans* and *E.coli.* The presence of lignin content in the Yew tree bark has been reported by previous researchers. According to the researchs of Cho et al., 2001; Erdemoglu et al., 2003; Cown, 1999, plants have Antimicrobial activities due to the content of lignans and flavonoid phytochemicals. They are most important groups of phenolic compounds.

5.3 Qualitative Test for phenol

The extract obtained from the bark of t*axus baccata* L. with 0.3 %HCl in methanol and distilled water was examined by using the special qualitative tests of phenol. The resulted data are tabulated in table (5.3).

Table (5.3) the Results of Qualitative Test for phenol

No	Experiment	Observation	Inference
1.	Acid Property Test	Blue litmus paper turns red	Phenol may be present
2.	Color with $FeCl_3$	Brown color was observed	Phenol is present
3.	Ellagic Acid Test	Muddy or nigger brown precipitate was appeared	Phenol is present

The result of the present study showed that the extract of the selected sample, which consists of phenolic compounds which exhibited the antioxidant activity.

5.4 Quantitative Determination of Total Phenolic Content

The total phenolic content of the bark of *taxus baccata* L. was determined spectrohphotometrically according to the Folin-Ciocalteu colorimetric method using gallic acid as the standard. The Folin-Ciocalteu method is a rapid and widely used assay to investigate the total phenolic content. This method measures the amount of the extract needed to inhibit the oxidation of the Folin–Ciocalteau reagent (Kale, A., et alJ. 2010)..Folin-Ciocalteau reagent is sensitive to reducing compounds including polyphenols. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce a blue coloured complex complex, the molybdenum blue which is measured at 765 nm colorimetrically. This blue colour complex can be quantified spectrophotometrically, thus total phenolic content can be determined (Pękal, A. and Pyrzynska, K. 2012). The absorbances of prepared sample solutions ((2 µl with ml distilled water) were measured with UV- visible spectrophotometer at 765 nm with respect to the blank solution for two times. The results are described in Table (4.1 and 4.2). From these results, the amount of total phenolic content of analyzed samples was obtained by using the standard graph. The content of total soluble phenolics of the bark of *taxus baccata* L. was expressed as gallic acid equivalent and it was 43.21 mg of gallic acid equivalent per 100 g dry weight.

According to the research of Md. Hasanuzzaman et.al, 2013, it is mentioned that the phytochemical screening of the extractives revealed the presence of flavonoid, tannin and phenol. Polyphenolic compounds, like flavonoids, tannins and phenolic, usually found in plants have been reported to have multiple biological effects, including antioxidant activity. Flavonoids and tannins present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action in the tested models.

6. Conclusion

The preliminary phytochemical test showed that the bark of *Taxus baccata* L. consisted of glycoside, flavonoid, polyphenol, phenolic, sugar, saponin, terpene, alkaloids, tannin, steroid and lipophenol compounds like flavonoids and tannins. Phenolic compounds have multiple biological effects including antioxidant activity.

Flavonoids and tannins may be responsible for the antioxidant action in the tested models.

Ethanol and ethyl acetate extracts of the bark of *T. baccata L.* showed the antimicrobial activities on all tested organisms. It was found that the total pheolic contents of the bark of *T. baccata* L. is 43.21 mg gallic acid equivalent per 100 g dry.

Thus, *T. baccata* L. can be regarded as natural plant sources of antioxidants.

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