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Research Article

Quantitative Pre-Eliminary Phytochemical Screening of Aqueous Extracts of Leaves of *Oroxylum indicum* from Five Different Places in Sumatra Island, Indonesia

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ABSTRACT
In this present study, we investigate quantitatively some phytochemical compound from the aqueous extracts of leaves of Kapung (*Oroxylum indicum*). The phytochemical compound that we investigated in this study is flavonoid, steroid, tannin, alkaloid, and saponin. The leaves of *Oroxylum indicum* were obtained from five different places in Sumatra Island, Indonesia. The results revealed that the leaves extracts contained all phytochemical that we investigated in this present study. The phytochemical compound content varies, depending on the leaves sampling places. The results indicated that the aqueous extracts of leaves of *Oroxylum indicum* contain some phytochemical constituents which can be beneficial for further investigation.

Keywords: Leaves, *Oroxylum indicum*, Phytochemical.

INTRODUCTION

The progress of the medical world enables the discovery of modern medicine for the treatment of various diseases. Although there are increasing number of effective drugs, public are still interest with traditional herbal medicine[1,2]. For this method of medicine, medicinal plants have been used for thousands of years. However, their activities proven with scientific methods, which can be employed to give a better understanding of their mechanisms of action, were only established in recent times[3]. Indonesia is an archipelago consisting of approximately 17,508 islands[4]. Among these islands, Sumatra is the one of the largest island in Indonesia, and the sixth largest islands in the world, with a total area of about 476,000 km², approximately 1800 km long, and 400 km wide. The island contains more than 10,000 plant species: the majority of which are found in lowland forest[5]. One of the potential plants that grow in Sumatra island is Kapung (*Oroxylum indicum*)[6]. *Oroxylum indicum* is a small-medium size deciduous tree widely distributed in tropical and subtropical regions. The plant is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan, South China, and South East Asia, including Indonesia[6,7]. In Indonesia, it is distributed in Java, Sumatra, Kalimantan, Sulawesi, Nusa Tenggara, and Maluku. This plant found in primary and secondary forests or in open areas, at an altitude of 1-800 m above sea level. This species is endangered because almost all parts of the plant are often used as pharmaceuticals or other purposes without followed by cultivation[8]. *Oroxylum indicum* has been used for centuries as an important herbal medicine in many Asian countries. In Indian Ayurvedic medicine, the root bark, stem, and leaf are prescribed for snake bite, diarrhea and dysentery. In Traditional Chinese medicine, the seeds of the plant, which are named Mu Hu Die in Chinese, have been widely used for the treatment of a cough, bronchitis, pharyngitis, pertussis and other respiratory disorders[9]. In Indonesia, the seeds of the plant are used to treatment bronchitis, costal pain, pharynx and laryngitis, and upper abdominal pain, the stem bark is used to treatment hepatitis, arthralgia, plant appetite, urinary tracts infection, and abdominal pain, and the root bark are used to treatment dysentry and diarrhea[11]. In south Sumatra, the leaves of *Oroxylum indicum* were used by Saling Tribes to treat jaundice and asthma bronchial. Its been widely accepted that the medicinal properties of plants are because the phytochemical constituents contained in the plant. The properties of these phytochemicals have been under investigation since the 1850s and they have been used as dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfumes, and even as drugs[12]. Several reports indicated that different parts of *Oroxylum indicum*, such as seeds, leaves, and bark of stem and root are known to contain substantial amounts of phytoconstituents such as phenolics, flavonoids, and tannins[13]. Also, from previous research, four flavonoid

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constituents from the seeds of *Oroxylum indicum* have been isolated and identified as chrysin, baicalein, baicalein-7-Oglucoside and baicalein-7-diglucoside (Oroxylin- B)[14]. Although some studies have shown that some parts of the *Oroxylum indicum* contain some phytochemical compounds, but there are still some limitations of the data, especially on the leaves of plants. In addition, research on this plant is also widely practiced in India and in other countries. Research on this plant in Indonesia is still limited. It promotes us to evaluate the phytochemical content of leaves of *Oroxylum indicum*.

MATERIAL AND METHODS

Samples collection

The leaves of *Oroxylum indicum* were collected from five different areas in Sumatra island, Indonesia. The areas of sampling were; (1) Kampang village, Seluma district, Bengkulu province with GPS coordinate 04º08'42,8" 102º40'30,8" and the altitude is 87 m above sea level; (2) Kungkilan village, Ogan Komering Ulu district, South Sumatra province with GPS coordinate 04º15'21,6" 104º06'19,2" and the altitude is 113 m above sea level; (3) Pahmungan village, Pesisir Barat district, Lampung province with GPS coordinate 05º10'38,3" 103º57'12,9" and the altitude is 57 m above sea level; (4) Gandasuli village, Liwa district, Lampung province with GPS coordinate 04º55'45,7" 104º01'37,6" and the altitude is 608 m above sea level; and (5) Baturaja village, Bengkulu Tengah district, Bengkulu province with GPS coordinate 03º42'03,3" 102º21'52,5"and the altitude is 54 m above sea level. *Oroxylum indicum* leaves were collected in April 2016. The collected leaves were air-dried in darkness at room temperature. Then, the dried samples were made into coarse powder using a commercial blender.

Methods of sample preparation

The applied method for the extract preparation was decoction extracting by boiling plant material. 100 g of the shade-dried of each leaf were boiled for 30 min in 1000 ml of distilled water. Then, the mixtures were allowed to cool to room temperature and filtered through Whatman no. 5 paper. The resulting solutions then used for the experimental protocol section.

Flavonoid content analysis

Total flavonoid content was determined by Aluminium chloride method using quercetin as a standard. 1ml of a test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Each experiment is carried out in triplicate and were expressed as percent (%) of flavonoid in leaves extract[15].

Steroid content analysis

1 ml of Methanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20ºC for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank. Each experiment is carried out in triplicate and were expressed as percent (%) of steroid in leaves extract[15].

Tannin content analysis

Tannin content was determined by Van-Burden and Robinson method. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min. Each experiment is carried out in triplicate and were expressed as percent (%) of tannin in leaves extract[16].

Alkaloid content analysis

The alkaloid content was determined by Harborne method. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. Each experiment is carried out in triplicate and were expressed as percent (%) of alkaloid in leaves extract[16].

Saponin content analysis

The leaves extract was dissolved in 80% methanol, 2 ml of Vanillin in ethanol was added, mixed well and the 2 ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600ºc for 10 min, absorbance was measured at 544 nm against reagent blank. Each experiment is carried out in triplicate and were expressed as percent (%) of saponin in leaves extract[15].

Data analysis

The results were expressed as mean±SE for three replicates. The data was analyzed using Microsoft excel 2010.

RESULTS AND DISCUSSION

The present investigation has been carried out to determine some phytochemical compounds present in aqueous extracts of the leaf of *Oroxylum indicum* obtained from several places on the island of Sumatra, Indonesia. The results are presented in figure 1-5. From figure 1, we can see that all plant extracts contain flavonoid, with the leaves extract from Gandasuli contain the highest amount of flavonoid and followed by the leaves extract from Kungkilan, Baturaja, Pahmungan, and Kampai. Flavonoids are low molecular weight bioactive polyphenols which play a vital role in photosynthesizing cells. The original "flavonoid" research apparently began in 1936, when Hungarian scientist Albert Szent-Gyorgi

was uncovering a synergy between pure vitamin C and as yet unidentified cofactors from the peels of lemons, which he first called "citrin," and, later, "vitamin P".[17] Till know, over 8,000 varieties of flavonoids have been identified[18]. Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and B) linked via a heterocyclic pyrene ring (C). They can be divided into a variety of classes such as flavones (e.g., flavone, apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin), and others[19]. Flavonoid have some biological effects such as anti-hepatotoxic, anti-inflammatory, and anti-ulcer activity. This compunds also inhbits several enzymes, such as aldose reductase, cyclooxygenase, Ca^{2+}ATPase, xanthine oxidase, phosphodiesterase, and lipoxygenase. These compound is a potent antioxidant and has free radical scavenging, and chelating metal activities[20-22]. The basic mechanism of these compound are hydrogen donation to another compound, and scavenge radicals by an H-atom or electron transfer process. This activity could inhibit the formation of another free radical, thus terminating the chain reaction[23]. Figure 2 represented the steroid content in aqueous extract of leaves of *Oroxylum indicum*. The result revealed that the highest steroid content is in the leaves extract of *Oroxylum indicum* from Kungkilan followed by Kampai, Gandsauli Pahmungan, and Baturaja. Steroid is terpenoid lipids characterized by the sterane or steroid nucleus: a carbon skeleton with four fused rings, generally arranged in a 6-6-6-5 fashion. Steroid varies by the functional groups attached to these rings and the oxidation state of the rings. The specificity of their different biological actions is due to the various groups attached to a common nucleus. When alcohol groups (OH) are attached, steroids should properly be called sterols (e.g., cortisol), whereas ketone groups (C=O) make them sterones (e.g., aldosterone)[24]. Steroids are partly responsible for the anti-diarrheal activity. The mechanism of action of some antifungal drugs is by binding to the cell membrane of pathogenic fungi in the presence of certain sterols,which subsequently disturb permeability and transport characteristics of the membrane,resulting in loss of intracellular cations[25]. Plant steroids have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness. Caution should be taken when using plant steroidal as small amounts would exhibit the much-needed stimulation on a diseased heart, whereas excessive dose may cause even death[26]. Figure 3 represented the tannin content in aqueous extract of leaves of *Oroxylum indicum*. The result revealed that the highest tannin content is in the leaves extract of *Oroxylum indicum* from Kungkilan followed by Baturaja, Pahmungan, Kampai, and Gandasuli. Tannins are polyphenols present in plants, foods, and beverages, and are of great economic and ecological interest. They are water soluble and with molecular weights ranging between 500 and 3000 Daltons. They also form complexes with water-insoluble proteins, alkaloids, and gelatin. They are responsible for the astringent taste of many fruits and vegetables, causing precipitation of salivary glycol-proteins and reducing oral lubrication[27]. Structurally, tannins possess 12–16 phenolic groups and 5–7 aromatic rings per 1,000 units of relative molecular mass. Hydrolysable tannins represent esters of phenolic acids (generally gallic acid as in gallotannins or other phenolic acids derived from the oxidation of galloyl residues as in ellagitannins) and a polyol, usually glucose. The galloyl groups can be further esterified or oxidatively crosslinked to yield more complex hydrolysable tannins[28]. According to their chemical structure and properties, tannins are divided into two main groups: hydrolysable and condensed tannins. Hydrolysable tannins (gallotannins and ellagitannins) are molecules which contain a carbohydrate, generally, D-glucose, as a central core, and condensed tannins are molecules which contain flavonoid units (flavan-3-ol) linked by carbon-carbon bonds[29]. Tannins have been implicated with various pharmacotherapeutic effects[30]. Tannins, in the form of proanthocyanidins, could have a beneficial effect on vascular health. Topical applications of tannins help to drain out all irritants from the skin. They are useful as an anti-inflammatory agent and in the treatment of burns and other wounds based on their anti-hemorrhagic and antiseptic potentials. In particular, tannins-rich remedies are used as anthelmintics, antioxidants, antimicrobials and antivirals[31], in cancer chemotherapy and to chelate dietary iron[32]. Figure 4 represented the alkaloid content in aqueous extract of leaves of *Oroxylum indicum*. The result revealed that the highest alkaloid content is in the leaves extract of *Oroxylum indicum* from Kampai, and followed by Gandasuli, Baturaja, Pahmungan, and Kungkilan. Alkaloid constitutes an important class of structurally diversified compounds. The term ‘alkaloid’ was coined by the German chemist Carl F. W. Meissner in 1819 and the word is derived from the Arabic name al-qali that is related to the plant from which soda was first isolated. These compounds are low molecular weight structures and form about 20 % of plant based secondary metabolites[33]. Alkaloid are characterized by great structural diversity, the presence of a basic nitrogen atom being the only unifying feature. Most alkaloids possesses just one nitrogen atom, but some have up to five. This nitrogen may occur in the form of a primary amine (RNH_2), a secondary amine (R_2NH) or a tertiary amine (R_3N). In addition to carbon, hydrogen, and nitrogen, most alkaloids contain oxygen. Alkaloid can occur as monomers or they may form dimers (also known as bis alkaloids), trimers or tetramers. Such oligomers are typically homooligomers, but heterooligomer also occur[34]. Alkaloid has influenced the human history profoundly due to their wide range of physiological effects on animals and pharmacological properties such as antibiotic, anticancer along with their potential exploitation as narcotics, poisons, and stimulants[33]. Moreover, several alkaloid exhibit significant biological activities, such as the relieving action of ephedrine for asthma, the analgesic action of morphine, and the anticancer effects of vinblastine. In fact, alkaloid is among the most important active components in natural herbs, and some of these compounds have already been successfully developed into chemotherapeutic drugs, such as camptothecin, a famous topoisomerase I (TopI)

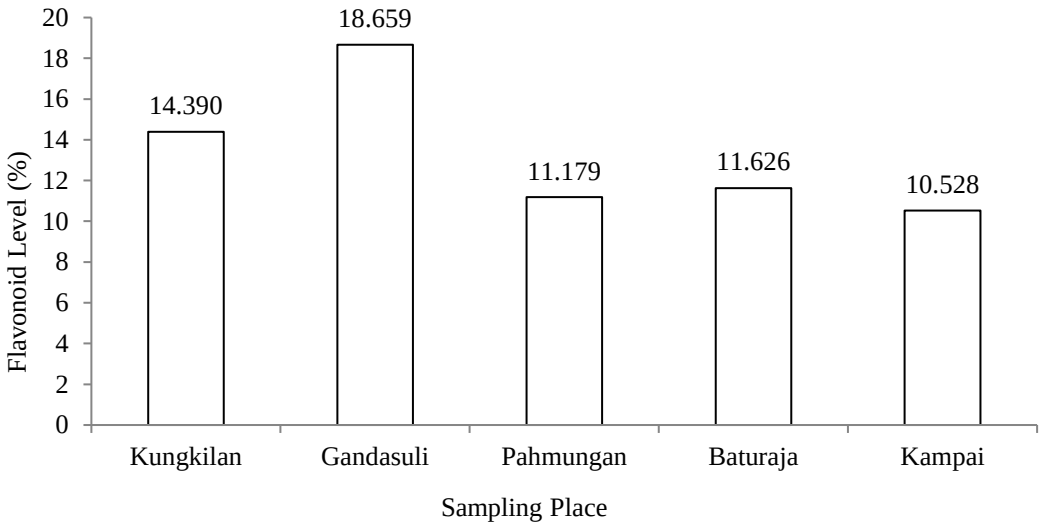

Figure 1: Quantification of flavonoid in aqueous extract of *Oroxylum indicum* from some different places in Sumatra, Indonesia.

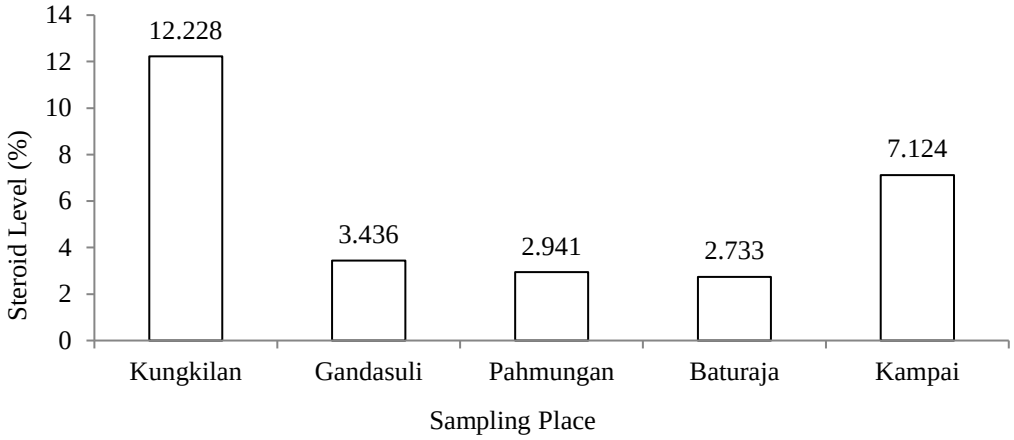

Figure 2: Quantification of steroid in aqueous extract of *Oroxylum indicum* from some different places in Sumatra, Indonesia.

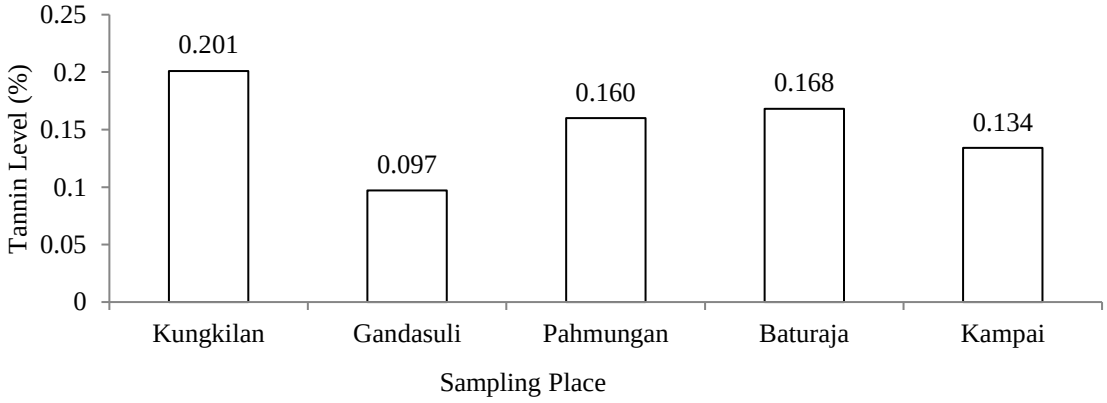

Figure 3: Quantification of tannin in aqueous extract of *Oroxylum indicum* from some different places in Sumatra, Indonesia.

inhibitor, and vinblastine, which interacts with tubulin[35]. Figure 5 represented the saponin content in aqueous extract of leaves of *Oroxylum indicum*. The result revealed that the highest saponin content is in the leaves extract of *Oroxylum indicum* from Kampai, and followed by Gandasuli, Baturaja, Pahmungan, and Kungkilan. Saponins are compounds with 'soaplike' behavior in water, i.e. they produce foam upon shaking. On hydrolysis, an aglycone is produced, which is called sapogenin. Saponins are extremely poisonous, as they cause

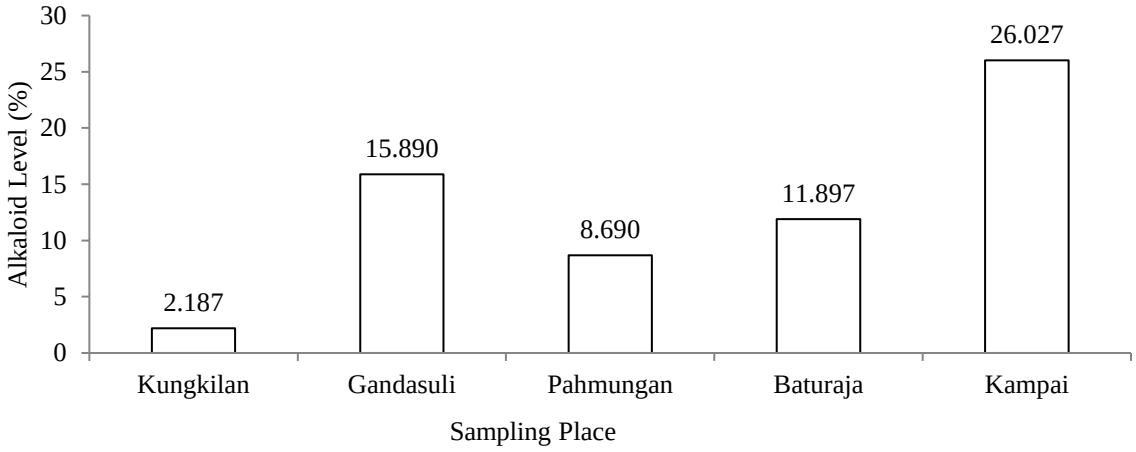

Figure 4: Quantification of alkaloid in aqueous extract of *Oroxylum indicum* from some different places in Sumatra, Indonesia.

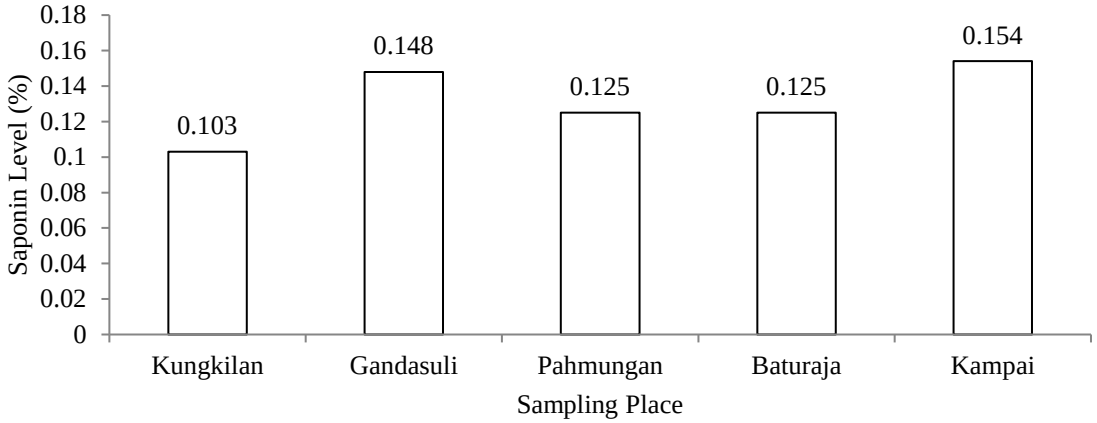

Figure 5: Quantification of saponin in aqueous extract of *Oroxylum indicum* from some different places in Sumatra, Indonesia.

haemolysis of blood and are known to cause cattle poisoning. They possess a bitter and acrid taste, besides causing irritation to mucosal membranes[36]. Chemically, saponin is triterpenoid, steroidal, or steroidal glycoalkaloids molecules that have one or more sugar chains in their structure[37]. Saponin has been reported to have a wide range of pharmacological and medicinal activities. It has been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs. It has been revealed that saponin has both hypertensive and cardiac depressant properties. They have been found to be potentially useful for the treatment of hypercholesterolemia, and diabetes. In addition, they have been reported to have antinematicidal, molluscicidal, insecticidal and antioxidant properties, anti-cancer agents, aphrodisiac properties, antiprotozoal effects, antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer effects[38]. In conclusion, the present study indicated that the aqueous extracts of leaves of *Oroxylum indicum* contain some phytochemical constituents, such as a flavonoid, steroid, tannin, alkaloid, and saponin. In addition, the levels of these compounds were found to differ, depending on where the plant sampling. Since the aqueous extract contains some phytochemical constituents it can be considered beneficial for further investigation.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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