

# Phytochemical Screening and Detection of Antioxidants Using the DPPH Assay of *Costus speciosus*: A Systematic Review.

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## ABSTRACT

Medicinal plants are garnering widespread interest in scientific circles due to their consistent pharmacological activities, which make them beneficial in the treatment of a variety of ailments. *Costus speciosus*, commonly known as crepe ginger, is an herbaceous plant native to Mexico that belongs to the family Costaceae (zingiberaceae). Creep Ginger is known to have many medicinal effects, such as antioxidant, anti-inflammatory, and potential antidiabetic activities. The aim of this review was to investigate the phytochemical compounds and potential antioxidant activity in *Costus speciosus*. The following information on the phytoconstituents of the species *Costus speciosus* was acquired from online scientific databases using NCBI, Pubmed, Google Scholar, Science Direct, Molecules, Elsevier, and ResearchGate searches. Phytochemical analysis from several studies showed the presence of alkaloids, flavonoids, steroids, phenolic compounds, tannins, terpenoids, and saponins in the extract. The parts of *Costus speciosus* that are used as medicinal plants are usually the leaf, rhizome, and roots. The results suggest that the presence of a wide range of phytochemical compounds from extracts of *Costus speciosus* may be used to estimate potential antioxidant activity using the DPPH methods. Extracts with methanol as a solvent show that DPPH has considerable antiscavenging activity and the present study on alkaloids indicates that their contents are responsible for the high antioxidant activity. This review study may help future research activities on *Costus speciosus* by providing up-to-date information and relevant data.

**Keywords:** *Medical Plant, Costus speciosus, Phytochemical Compounds, Antioxidant activity, DPPH assay, EC<sub>50</sub>, IC<sub>50</sub>.*

## 1. INTRODUCTION

Medical Plants often play vital roles in human health and it is known to contribute to many kinds of disease treatment. The study of using medical plants to create a pharmacologic effect could be identified as herbal medicine [21]. Herbal Medicine is created with the intention of enhancing human health and minimizing the progress of disease. There are many kinds of medical plants that have been used further as herbal medicine like ginger, ginseng, dan creep ginger. Medical plants contain many metabolites that play a vital role in

inhibiting many progressive diseases thus giving them a remark on their contribution for many kinds of disease treatments [4]. Metabolites is a product that can perform many biochemical reactions and some of these reactions can carry a pharmacological effect that herbal medicine desires [7] ; [39]. The metabolites that are available in the medical plants could be categorized as primary and secondary metabolites [2] ; [6]. Primary metabolites are essential types of metabolites that participate directly in development of the plant itself while secondary metabolites are a more of a product to the plant itself rather than a contributing factor to its own growth [30]. Some examples of primary metabolites are carbohydrates and hormones while secondary metabolites consist of polyphenols, flavonoids, steroids, alkaloids, terpenoids, and tannins [27].

Phenolic compounds are one of few secondary metabolites that present in a variety of plant parts. This secondary metabolites display an activity of antioxidant and antiinflammatory. The uniqueness of phenolic compounds could be seen in its possession of an aromatic ring composed of at least one hydroxyl substituents. This could be divided into several groups like flavonoids, tannins or phenolic acid [37, 38]. Flavonoids are compounds that have a phenolic structure composed of polyphenol rings. Flavonoids are categorized as a part of ketone group and have a hydroxyl group in position C-3 in C ring [5]. Flavonoid compounds consists of polyphenolic compounds with the presence of benzo- $\gamma$ -pyrone structure [32]. Flavonoids are categorized as hydroxylated phenolic with a considerable kinds of pharmacological activities. Flavonoids are divided into a few parts like chalcone, anthocyanins, anthocyanins, isoflavones, flavones, flavonols, and flavones. These secondary metabolites have a broad spectrum in pharmacological effect as it promotes anti-inflammatory, antioxidant, and antidiabetic. Flavonoids can be found in many parts of the plant like leaves, woods, flowers, fruits, and roots [33]. Flavonoids itself play many important roles in biological activities [34]. One of its effects could protect skin interaction from UV lights as well as improving skin barriers [3].

Saponins are glycoside compounds that can be found in many types of plant. Saponins themselves have a big molecular weight composed of aglycon from either steroids or triterpenoids [36]. Saponins have a positive effect that functions as antioxidants. Many types of research show that secondary metabolites have a lot of positive effects, however the usage of saponins needs to be limited because it does contain negative effects on certain uses if not controlled well [24]. Saponins are soluble in water but can be easily affected by thermal. This makes the screening of saponins needs adjustment on the temperature. Steroids are compounds which are composed of four fused rings and are categorized by a few types [35]. Some of them serve as functional hormones which for example like estrogen, progesterone, corticoids, and androgen. Estrogen mostly plays a huge role in female hormones while androgen plays a huge role in men hormone. Corticoids are mostly used in suppressing inflammation like anti-inflammatory drugs. Terpenoids are metabolites secondary consist terpenes with the addition of oxygen group and structure reformation. This secondary metabolite is usually divided based on the classification of the carbon number they possess. Monoterpenoids for ten carbon, diterpenoids for twenty of carbon, and so on. Terpenoids with 40 or higher numbers of carbon could be identified as polyterpenoids. Alkaloids are compounds that contain nitrogen atoms and are basic to extract which requires the addition of hydrochloric acid. Addition of hydrochloric acid Aims to extract alkaline alkaloids using an acidic solution [24]. Alkaloid testing can be done using 3 reagents, namely mayer, dragendorff, and bouchardat. The positive results of alkaloid compounds in Mayer's reagent are indicated by formation of a white to yellowish precipitate. Alkaloid compounds will interact with ions tetraiodomercurate (II) to form complex compounds and precipitate. Tannin are compounds that are polyphenolic and often found in higher plants. The presence of polyphenolic structure are necessary but not the only factor that categorized it as the basic rule of joining tannins structures. The galloyl glucoside that tannins contain is not the sole primary requirement for the tannin classification either, however the residue of galloyl glucoside can link to another residue and create even more unique diverse structures [24].

Creep ginger or *Costus speciosus* is an herbal medicine that belongs to the Costaceae (zingiberaceae) [14]. *Costus speciosus* origin comes from Mexico but is cultivated through certain South Asian regions. *Costus speciosus* is a plant that can reach around 2,5 m in high and consist of knurl root, large base flowers, a solid white color flower with broad size bloom figure, a cone-like stem with spikes around it which are much thicker than a typical rose [9] ; [28]. Although the appearance can differs by slight different based on the regions distribution [4]. Nowadays, this plant can be found vastly in Indo-Malayan area and Sri-Lanka. *Costus speciosus* is bred by vegetative rhizomes in which it will go on for a few cycles. The bred will happen through the warm season in the region of the plant, mostly in March and can last until October. During March and April, the plant will enter the growing stage which means the production of new parts of the plant like leaves, roots, or stems will take place. Once the warm season is over, the plant will be dormant until suitable conditions are met [6]. From many phytochemical screening research, *Costus speciosus* usually contains flavonoids, tannins, polyphenols, steroids, and phenolic compounds in their structure. These secondary metabolites will later play a part in the pharmacological activities [29].

The family of Zingiberaceae consist of more than a thousand species of plant and many of them are distributed in tropical and subtropical areas. *Costus speciosus* can be found in many countries like Malaysia, Indonesia, South China, India, Vietnam, etc [52]. There are many species of Zingiberaceae plants that make them particularly potent as possible herbal remedies. Upon further studies, it is known that creep ginger has many medical effects, like antioxidant, anti-inflammatory, and potential antidiabetic activity. The classification of *Costus speciosus* could be identified as follows:

**Table 1. Taxonomic classification**

<b>Botanical Name</b>	<b><i>Costus speciosus</i></b>
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Costaceae
Genus	Costus
Species	Speciosus

Sipika et al., 2015 [25]; Srivastava, 2011 [26]

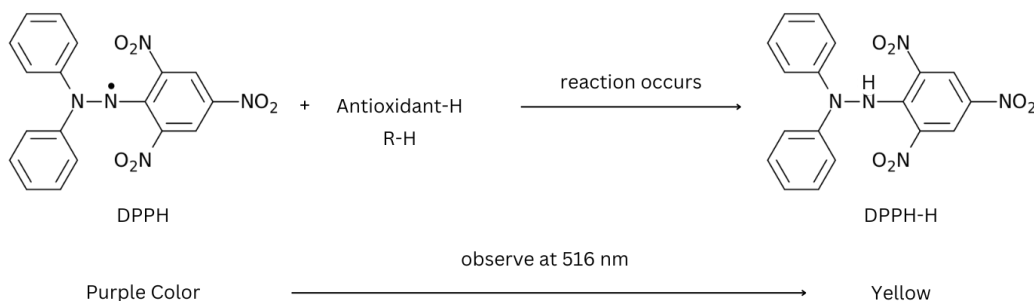
The process of detecting secondary metabolites is defined as phytochemical screening. Phytochemical screening is an important procedure in order to study plants for its structure and effects [13]. Phytochemical screening is an efficient method to study plants because this test could be performed on many parts of the plant. The test could use and identify metabolites from leaf, fruit, rod, flower, and roots. The procedure of phytochemical screening could be done by adding different reactions and different treatments in each of its metabolite identification [21]. The identification will be able to detect many kinds of secondary metabolites. For example the detection for tannin could be performed using drops of ferric chloride and the presence of tannin could be identified by changing color especially if the occurrence is a blackish color. Flavonoids test could be performed using hydrochloric acid or ammonium chloride in which the presence of flavonoids could be shown by the occurring

pink or red color. The method of flavonoids detection is not limited to only using hydrochloric acid or ammonium chloride. Another way to detect flavonoids could be detected using a plant residue in with the addition of ethanol followed by either  $H_2SO_4$  or  $NaOH$  to the residue. A greenish-yellow color indicates positive flavonoids in the  $H_2SO_4$  reaction, or a blue-purple color indicates positive flavonoids in the  $NaCl$  reaction [24]. The phytochemical screening of *Costus speciosus* is important because the potential effect of what it could give depends on the secondary metabolites this plant could offer. A different extract of the *Costus speciosus* itself will determine a different secondary metabolite it could contain thus giving a diversity of effect it could potentially offer [23].

*Costus speciosus* is known for its antioxidant effects. It is reported that *Costus speciosus* has the necessary secondary metabolites to respond to said activities through the abundance of alkaloids, flavonoids, saponins, and steroids. It is known that up to at least 20% of plant species contain alkaloids. One of the tests to measure the correlation of secondary metabolites with its activity of antioxidants is through the essay of DPPH radical scavenging. The 2,2 diphenyl 1-picrylhydrazyl or known as DPPH radical scavenging assay is a method to calculate the estimation of antioxidants based on the color change on the test sample. The DPPH radical is a stable radical free that has a deep purple color and the ability to interact with an odd electron in a certain wavelength [40].

The DPPH in the oxidized form will react with antioxidants (R-OH) resulting in decolorization of the original purple color with the emergence of yellow color. This happens because the antioxidant will react with a certain different possible mechanism which consist of HAT (hydrogen atom transfer) or SET (single atom transfer) [53]. the reaction could be observed as [54]:

Fig 1. Chemical transformation



Liang and Kitts, 2014[31]; Hairunisa,

2020[53]

## 2. EXPERIMENTAL DETAILS

In preparing this article review, the authors have used references from research articles relating to the result of phytochemical screening and antioxidant activity in *Costus speciosus* reported in the last ten years. The search was carried out through electronic databases from various sites of journal publications, such as NCBI, Pubmed, Google Scholar, Science Direct, Molecules, Elsevier, and ResearchGate, and others. The data sources used consisted of international journals as the main data source and national journals from Indonesia as additional data sources. From the several references obtained, screening and selection of references were carried out. Selection of references used by making exclusion criteria and inclusion criteria. The literature included in the inclusion criteria contained information about *Costus speciosus* and their metabolite content and antioxidant activity. While the literature included in the exclusion criteria did not contain information about *Costus speciosus* with antioxidant activity.

## **2.1. Inclusion Criteria**

In selecting the source article, the authors used few references such as *costus speciosus* which consists of its taxonomy, details, characteristic, phytochemical compounds, and addition of antioxidant effect that it possesses. The antioxidant articles are focused on the method of DPPH-assay but not limited to its variation. The authors also attached its references about methods that performed such as the identification of its secondary metabolites which were limited to alkaloids, saponins, flavonoids, tannins, sterpods, terpenoids, phenolic compounds.

## **2.2 Extraction Methods**

Methods to prepare extract could be done with maceration, soxhlet, percolation, ultrasound, alcohol extraction, or counter-current extraction. The extraction methods are not limited to those methods only. However, the most common extraction methods are maceration, soxhlet, and percolation. Maceration extraction relies on the process of crushing, which, after the grinding process, is placed in a flask. A solution (mostly ethanol) was then added to soak them for a certain amount of time. Soxhlet methods solely rely on the soxhlet apparatus. The process of extraction is later done by creating a flow of solvent that comes into contact with the sample. This process will continue for a specific repeatable cycle until a few hours have passed, with a minimum of 16 cycles performed. Percolation is an extraction method that relies on the flow of solvent just like the other methods; however, the sample has already interacted with the solvent before the flow begins, which differs from soxhlet methods. The sample is placed on a cylinder flask with pores, and then the solvent will be placed above. Factors that affect the content of secondary metabolites in plants can be influenced by the selection of samples, the selection of solvents, and the techniques used. The sample selection factors consist of the site of collection, environmental factors, and plant development, while for the solvent selection factor, the solvents that can be used are Chloroform, Ethanol, Methanol, Water, Methanol, Acetone, and Ether. Besides that, the extraction method used also plays an important role in the process of isolating metabolites in plants, where the methods used can be carried out with Maceration, Percolation, Soxhlet, Infusion, and Ultrasound.

## **2.3 Phytochemical Screening**

### **2.3.1 Alkaloids**

The identification process of alkaloids begins with the addition of aqueous HCl and then followed by the addition of a few drops (5 drops) of dragendorff. After the sample is added, it is filtered and analyzed visually. An indication of a change in color will determine the presence of alkaloids in the sample. The positive presence of alkaloids could be deduced from the occurrence of orange-to-red precipitation.

### **2.3.2 Saponin**

The identification process of saponin is done by using distilled water, which is then filtered with whatman paper into a flask. The sample is then shaken a few times. The indication of saponin will show the formation of bubbles around the flask.

### **2.3.3 Flavonoids**

The identification of flavonoids is performed by adding ammonium chloride to the extracts. The indication of flavonoids could be analyzed from the color change. The formation of a yellow-colored solution will indicate the presence of flavonoids in the extracts.

### **2.3.4 Tannin**

The identification of tannin is performed using the ferric chloride drops. The sample (powder) is prepared using the same methods that is done for the identification of saponin (distilled water and whatman filter). The indication of flavonoids could be analyzed from the color change. The formation of dark green (brownie) or dark blue indicates the presence of tannin in the sample.

### **2.3.5 Steroids**

The identification of steroids is done by adding methanol and chloroform to the sample, followed by addition of sulfuric acid. The indication of flavonoids could be analyzed from the color change. The formation of combination red in the upper section and yellow-green in lower section indicate the presence of steroids.

### **2.4 Determination of Antioxidant Activity**

The determination of antioxidant activity could be estimated using the DPPH methods or which is known as 2,2-diphenyl-1-picrylhydrazyl assay. The reaction of DPPH radical will indicate the presence of antioxidant activities through color change reaction in which the occurrence of yellow color indicates the activity. Although other methods could be used depending on the research, the DPPH assay gives accurate estimation and has been performed through a lot of recent studies.

### 3. RESULT AND DISCUSSION

Table 2. Phytochemical compounds in extract of *Costus speciosus*

Solvent used for Extraction	Plant Part	Phytochemical Compounds							References
		Alkaloid	Saponin	Flavonoid	Tannin	Steroid	Terpenoid	Phenolic	
Methanol	Leaves	-	+	+	-	-	+	+	[1]
Ethanol	Leaves	-	+	+	+	-	+	+	[1]
Ethanol	Leaves	+	-	-	-	+	-	+	[18]
Methanol	Leaves	+	+	-	+	-	+	+	[16]
Methanol	Leaves	-	-	-	-	-	+	-	[9]
Ethanol	Leaves	+	+	+	+	+	+	+	[11]
Methanol	Leaves	-	+	+	+	-	-	-	[12]
Ethanol	Leaves	+	+	+	+	-	-	-	[14]
Methanol	Leaves	+	+	-	-	+	-	-	[49]
Methanol	Leaves	-	-	+	-	-	-	+	[15]
Methanol	Leaves	+	-	+	-	+	+	+	[20]
Methanol	Leaves	+	+	-	+	-	+	+	[42]
Ethanol	Leaves	-	+	+	+	-	+	-	[43]
Ethanol	Leaves	+	-	+	+	+	+	+	[47]
Methanol	Leaves	+	+	-	+	+	-	+	[48]

Solvent used for Extraction	Plant Part	Phytochemical Compounds							References
		Alkaloid	Saponin	Flavonoid	Tannin	Steroid	Terpenoid	Phenolic	
Ethanol	Leaves	+	+	+	+	+	+	+	[51]
Methanol	Rhizome	-	-	-	-	-	+	-	[9]
Methanol	Rhizome	-	+	-	+	+	+	-	[1]
Ethanol	Rhizome	-	+	+	+	+	+	-	[1]
Methanol	Rhizome	+	+	+	+	-	-	-	[19]
Methanol	Rhizome	+	+	+	+	-	-	-	[16]
Methanol	Rhizome	+	+	+	+	+	-	N/A	[10]
Methanol	Rhizome	+	-	+	-	+	+	-	[20]
Ethanol	Rhizome	-	+	-	-	-	-	-	[41]
Methanol	Rhizome	-	+	+	-	+	-	-	[44]
Ethanol	Rhizome	+	+	+	+	+	+	-	[45]
Hydroalcoholic	Rhizome	-	+	+	-	-	-	-	[46]
Methanol	Stems	-	+	-	+	-	-	+	[1]
Ethanol	Stems	-	+	-	+	-	-	-	[1]
Ethanol	Stems	-	+	+	+	+	-	+	[18]
Methanol	Stems	-	+	+	+	-	+	-	[16]
Methanol	Stems	+	-	+	-	+	+	+	[20]

Solvent used for Extraction	Plant Part	Phytochemical Compounds							References
		Alkaloid	Saponin	Flavonoid	Tannin	Steroid	Terpenoid	Phenolic	
Methanol	Flowers	+	+	-	+	-	+	-	[1]
Ethanol	Flowers	-	-	-	-	-	+	-	[1]
Methanol	Seeds	-	+	+	+		-	+	[1]
Ethanol	Seeds	-	+	+	-	-	-	-	[1]
Methanol	Leaves, roots, rhizomes, and stem	+	+	-	+	+	+	+	[8]
Ethanol	Root & Tubers	-	+	-	-	-	-	+	[17]
Methanol	Tubers	+	+	+	N/A	N/A	N/A	+	[13]

\*N/A : not available, (+) : positive, (-) : negative

*Coctus speciosus* a plant with an abundance pharmacological aspect that plays a vital role in human medical plants. The plant itself belongs to the family of costaceae in which could be seen easily among the zingiberales, consisting of a unique branch that has the monistic character and is known for its antioxidant activity. One of the tests to measure the correlation of secondary metabolites with its activity of antioxidants is through the essay of DPPH radical scavenging. The indication of antioxidant activity will result in the decolorization that changes from deep purple by time after the exposure. This assay is simple and already widely used to estimate antioxidant activity. Although different methods could have a different estimation, the DPPH radical scavenging test produces a quite accurate estimation of antioxidant activity. Based on recent studies, the DPPH radical scavenging test is performed on 20 alkaloids compounds that show that most of them have a significant antioxidant activity even higher than from standart. Another method that estimates the antioxidant effect available is from the TAC method or total antioxidant capacity which focuses on the reduction process of Mo(VI) through the emergence of phosphate with a solid green color and the final product of Mo(V). The preparation of extract of *Costus speciosus* is done through variation of methods. The utilization of the right solvent is necessary to ensure the containment of therapeutic active components. The usage of alcoholic solvent like ethanol or methanol determines the presence of alkaloids, sterols, saponins, terpenoids accurately based on how long the extraction place is taken, the temperature, the solvent and sample ration, and the pH of the solvent that is used during the preparation. Ideally, the solvent needs to be non-toxic and can be vaporized on certain desired conditions. Beside alcoholic solvent, water is also a common solvent that utilizes the determination of saponins, tannins, terpenoids, anthocyanins, lectins, and polypeptides. Although it is determined that alcoholic solvents like ethanol and methanol are more potent to extract antioxidant activity substances than the water solvent. Another solvent that is available to use during the screening process are acetone, ether, and chloroform. Acetone is useful to screen flavonols and phenolic compounds more than the alcoholic solvent but in general the alcoholic solvent covers most of the secondary metabolites compared to acetone. Ether is useful for the extraction of fatty acids and coumarins while Chloroform is useful for the extraction of tannins and terpenoids. Based on the studied reference it shows that *Costus speciosus* has abundance contents of alkaloids, saponins, flavonoids, tannins, steroids, terpenoids, and phenolic compounds. The presence of these secondary metabolites could create several pharmacological activities like antimicrobial, anti inflammatory, and antioxidant.

**Table 3. Antioxidant Activity through the DPPH Assay**

References	Plant Parts	EC <sub>50</sub> Value (µg/mL)
Behera et al., 2020 (Methanol Extract)	Rhizome, leaves, and stem	Rhizome : 62.05 ± 0.03 Leaves : 53.05 ± 0.05 Stem : 50.05 ± 0.03
Vijayalakshmi and Sarada, 2008 (Methanol Extract)	Leaves, Stem, and Roots	Leaves : 139 ± 1.55 Peel of Stem : 84.4 ± 0.48 Peeled Stem : 315.3 ± 0.99 Root : 14.2 ± 0.55
References	Plant Parts	EC <sub>50</sub> Value (%)

References	Plant Parts	IC <sub>50</sub> Value (µg/mL)
Jagtap and Sapute, 2014	Rhizome	Rhizome : 78.03
Nehete et al., 2010 (Methanol Extract)	Rhizome	14.26 ± 0.88
Pizon et al., 2018 (Ethanol Extract)	Leaves	690
Kodagoda et al, 2023 (Methanol Extract)	Leaves	91.50 ± 0.08

From the determination of antioxidant activity, Behera et al. performed studies based on the origin of using DPPH solution, the sample extraction concentration (rhizome, leaf, and stem) is prepared 100 µg/mL and then 5 mL is used with the addition of 1 mL of 0,001% ethanolic solution of DPPH. The compounds used were incubated for around half an hour with the temperature of 30±2°C. The reading takes part at 516 nm wavelength using the spectrophotometer and then the result is analyzed. The result shows that the antioxidant (expressed as EC<sub>50</sub>) shows at least around 50 µg/mL for each sample part. This result indicates the sample used in this study shows a potential to be further used or studied in order to enhance the antioxidant activity. The used plant sample is obtained through the Khurda district, Chandaka-Dampara Wildlife Sanctuary. The DPPH method performed from Jagtap and Sapute is performed using the 0,1 mM DPPH solution mixed in methanol (0.0394 in 1000 mL methanol) and then the detection using HPLC (High Performance liquid chromatography). The result shows antioxidant activity of *Costus Speciosus* with 4 different extracts (water, methanol, chloroform, acetone). The methanol extract shows the highest activity with a total of 78.03% while acetone produces 30,02% and Chloroform produces 18,33%. The water extract follows lastly at 9.98% activity. The method performed on Nehete et al. is done through the addition of 1 mL of DPPH solution into variety concentration. The reaction is waited for 20 minutes and then the absorbance was recorded at 517 nm. The studies performed with Vijayalakshimi and Sarada were reported in different extracts (methanol, ethyl acetate, and chloroform) resulting in a variety. The methanol extract showed the highest result on peeled stem with the activity of 315,3 ± 0.99 expressed as EC<sub>50</sub> and the result of scavenging activity of total 57,96%. This resulting in the stem parts of *Costus speciosus* could potentially serve as a potent antioxidant that could replace synthetic antioxidants. The total antioxidant capacity (TAC) from research performed by Kodagoda et al. was determined using phosphomolybdate complexes formation technique from methanol leaf extract. The percent of DPPH radical inhibition of *C. speciosus* methanol leaf extract ranged from 91.50 µg/mL expressed as IC<sub>50</sub>. In a study performed by Pizon et al., hydroalcoholic *C. speciosus* leaf extract showed DPPH scavenging action. The activity value of 70% ethanol is 690 µg/mL expressed as IC<sub>50</sub>.

#### 4. CONCLUSION

This review summarizes the phytochemical compounds in *Costus speciosus* species. The phytochemical analyses of the extracts revealed the presence of secondary metabolites. A phytochemical analysis of *Costus speciosus* extract was carried out specifically to examine the content of alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and phenols. The selection of solvents is critical in the extraction of phytochemical compounds, and from

the results of the review, it can be concluded that the content of phytochemical compounds is more readily detected in extracts with ethanol solvent. According to the literature research, the compounds that showed the most antioxidant activity on *Costus speciosus* were alkaloids. Furthermore, as a versatile antioxidant agent, it appears to have potential. The authors hope the information provided in this review may help future research studies on *Costus speciosus*.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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