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Research article

Extraction and Phytochemical Profile of Three Herbal Weeds: *Chromolaena odorata* L., *Amaranthys virdis* L., and *Cyperus Rotudus* L. for Green Synthesis of Silver Nanoparticles

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Abstract: Phytochemical screening and silver nanoparticle synthesis of extract of 3 local weeds from Sakon Nakhon Province, namely, *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L., were carried out. The preliminary testing of chemical constituents revealed secondary metabolites with pharmacological activity. The weed extracts possessed bioactive compounds and were potent for silver nanoparticle synthesis. The weed extracts demonstrated a lower inhibition percentage (DPPH inhibition) than ascorbic acid, with significant differences at $p<0.05$. Reducing agent analysis revealed that the weed extracts contained reducing compounds such as phenol and carboxylic acid suitable for AgNPs. The XRD patterns of silver nanoparticles synthesized from the extracts demonstrated the efficacy of the weed extract for medical applications (the production of AgNPs). In the future, the size and morphology of silver particles should be investigated. The current results are expected to be a guideline for further applications of local weeds.

Keywords: Herbal weed; green synthesis; biological approach; Ag nanoparticle

1. Introduction

"Principles and Practices of Weed Management" proposed the meaning of weed as the type of plant which are unwanted, useless, and harmful. There are a variety of weed definitions, such as "a plant out of place or growing where it is not wanted" [1]. Weeds cause various problems, harming the global economy, particularly in agriculture, where they compete for nutrients, water, and light with the crop. Herbicides have been used to reduce the amount of weed in agricultural areas [2]. Nevertheless, weeds have important chemical constituents with pharmacological potential. The common weeds observed in the region of Sakon Nakhon province are *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L.

Chrmolaena odorata L. or Siam weed is commonly found in tropical Africa, North America, and South and Southeast Asia, as reported by biological action and the efficiency of antimicrobials [3]. *C. odorata* is a plant belonging to

the Asteraceae family (Aster family), Chromolaena genus (Thoroughwort), and the species of *Chromolaena odorata* L [4]. According to Srisuda *et al*. [3], the leaf, stem, and root of *Chromolaena odorata* were extracted using various solvents (water, ethanol, methanol, and hexane). That showed a correlation between total phenolic content and total flavonoid content. *Amaranthus virdis* L. or Amaranthaceae is a herbal that rapidly spreads in Asia, Africa, and Latin America. The extracts of amaranth leaves revealed total phenolic content of 1.03 to 3.64 GAE, g/100 g, total flavonoid content of 18.4 – 5.42 QE, g/100 g, and radical scavenging activity of IC_{50}:14.25 - 83.43 µg/ml [5]. *Cyperus Rotudus* L. is a plant [6] with the local name of Saed, Sajal, Seil in Arabic and nut grass, purple nutsedge in English, and Xiang Fu in Nagarmotha and China [7]. *C. rotundus* was used in traditional medicine recipes for its beneficial compounds, such as phenols, flavonoids, tannins, and glycosides, which have a medicinal effect [8].

Silver nanoparticles (AgNPs) are increasingly used in many fields due to their physical and chemical properties (in medicine, food, health care, consumer, and industrial applications). AgNPs, which have many benefits, can be used for antibacterial, food, household, health care, medical device products, optical sensors, and cosmetic products [9]. The AgNPs properties are based on aspects of the crystal (size of crystalline, density, and structure). The distribution of AgNPs nanoparticles inhomogeneous crystalline demonstrated high chemical and physical properties [10]. That can be synthesized using both physical and chemical processes. Physical procedures include laser radiation and condensation processes, while the chemical approach, which provides for hydrazine, sodium borohydride, and green synthesis, is the most widely used method [11]. Nanotechnology has been used in industrial agriculture to increase agricultural products and decrease postharvest waste. AgNPs have been applied to extend the life of farm products as ethylene inhibitors and antimicrobial agents [12].

Green synthesis is a process that can produce silver nanoparticles in cost-effective, environmentally friendly, and direct ways. Silver nanoparticles have been improved by using the silver solution and plant extracts with insoluble organic compounds [13]. The plant extracts converted the $AgNO_3$ solution to AgNPs, which improved their size, morphology, and optical properties [14]. MOSHFEGH *et al*. [15] synthesized nanoparticles of gold (Au), silver (Ag), and gold-silver (Au-Ag) by using biological synthesis. The results demonstrated maximum absorption at 530 nm for AuNPs, 440 nm for AgNPs, and 458 nm for Au/AgNPs. The laser light scattering method revealed the particle sizes of Au, Ag, and Au-Ag as 89 nm, 37 nm, and 63, respectively. The literature has reported advances in nanoscience and nanotechnology, both of which have medical applications. AgNPs are reported to possess therapeutic activities, such as antifungal, antiviral, antiinflammatory, and anticancer [9]. Siddhant and Mohan [16] synthesized AgNPs from *Ocimum Sanctum* (Tulsi) and its derivative quercetin (flavonoid was present in Tulsi). Various physicochemical conditions synthesized the AgNPs regarding pH, temperature, reaction time, and reactants concentration. The silver nanoparticles separately produced from leaf extract and neat quercetin demonstrated the same optical properties, morphology, and antimicrobial action.

According to the information on the properties of weeds, the following are *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L. That is pharmaceutical properties and the typical local plant. The biosynthesis process of the plant extracts can be used to synthesize silver nanoparticles. The nanoscale has multiple medicinal properties. This research proposed to use the local plant extracts of *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L. for AgNPs synthesis. That is cost-saving, reduces chemical waste, and is environmentally friendly. According to the results, to guide the weed usable for more applications.

2. Materials and Methods

2.1 Identification and Collection of The Three Herbal Weeds

The leaves of *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L. were obtained from Sakon Nakhon, Thailand. The specimens of the plants were identified and confirmed by three experts in botany, Thai pharmacy instructors, and plant science professors.

2.2 Preparation and Extraction of The Three Herbal Weeds

The fresh leaves of *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L. were cleaned with distilled water and desiccated. The plant samples' crude extracts were extracted using the decoction method (T_b = 100°C) [17]. A hundred grams of each sample of fresh weed leaves were chopped into smaller pieces. This was then boiled with 500 ml of distilled water (5 minutes) and filtrated with a filter paper (Whatman No.1). The crude solution was kept at 4°C for 7 days for sedimentation, after which the upper layer was collected. The filtrated solution was used for analysis and the synthesis of AgNPs.

2.3 Silver Nanoparticle Synthesis

The reaction for AgNPs synthesis was carried out with 100 mL of the sample with 900 mL of $AgNO_3$ (1mM) and treated with sodium hydroxide solution at pH 8 (1M). The mixture was heated at 70 °C for 1 h and then centrifuged at 11,000 rpm for 5 minutes. The obtained black powder was kept for analysis [17-18]. The plant extracts presented a phytochemical capable of donating electrons to reduce Ag^+ ions to Ag^0 [19]. Figure 1. shows the silver ion reduction, agglomeration, and stabilization to form a particle of nano size.

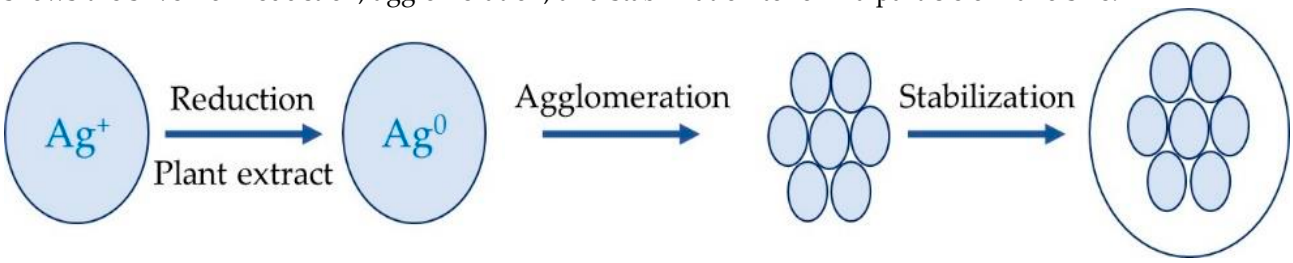

Figure 1. The mechanism of AgNPs synthesis.

2.4 Analysis of the three Herbal Weed Extracts and AgNPs

2.4.1. Preliminary Phytochemical Phytochemical Testing

Preliminary phytochemical testing was used to analyze the secondary metabolite samples of the weed extracts. The phytochemical screening focused on cardiac glycosides, flavonoids, saponins, tannins, terpenoids, anthraquinones, coumarins, phlorotannins, steroids, alkaloids, and glycoside (three replications) [20-22].

2.4.2 Gas Chromatography-mass spectrometry (GC-MS)

The screening of chemical substances in the weed extracts was carried out using the GC-MS technique, Shimadzu GCMS-QP2020 Ultra mass selective detector coupled with Shimadzu QP2020 Ultra gas chromatograph, equipped SH-Rtx-5MS capillary column (30 m x 0.32 µm, film thickness 0.25 µm). The GC-MS method was improved by Keshab *et al*. [23]. The sample was 0.2mg/mL, and helium gas was used as a carrier (flow rate: 3.0 mL/min). The initial column temperature was 50°C and then was programmed to increase at a rate of 5°C/min to 140°C (constant for 5 min), 140-200°C (3°C/min), and 200-260°C (15°C/min). The temperature was adjusted at 250°C for the injector and 280°C for the detector with a split ratio of 1:50. The electron impact voltage was set at 70 ev. The chemical constituents were identified by comparing their retention index with Library NIST17.

2.4.3 Determination of reducing agent

Metal nanoparticle synthesis requires a reducing agent. Most plants contain free radical scavenging molecules as reducing agents, which can be used to synthesize metal nanoparticles. [24]. The reducing agent was determined following the conventional method (Carboxylic acid testing and Amine testing) [25-26] and the modern method (FTIR spectroscopy analysis) [27]. The reducing agents were measured using FTIR (ATR-FTIR; Bruker TENSOR II) following the method reported by Piotr *et al*. [28]. The extracts were dried at 40°C for 7 days and placed on a sample holder. Four scans of dried extracts were measured from 600 – 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4.4 Scavenging activity percentage

The antioxidant percentage of the sample weed extracts was determined compared to standard free radicals (1,1 diphenyl-2-picryl-hydrazyl: DPPH). The DPPH radical scavenging activity was

evaluated following the research of Eugenio *et al.* [29] and was compared with standard antioxidants (ascorbic acid). The reaction for %AA determination consisted of 0.5 mL of sample, 3 mL of ethanol, and 0.3 mL of DPPH (0.5mM DPPH ethanolic solution). The antioxidant compound provided the hydrogen, which changed in the dark purple DPPH solution to yellow (after incubation for 30 minutes). The light absorption of the reaction was performed at 517 nm. A blank solution obtained from ethanol (3.3mL) and sample (0.5 mL) and ethanol (3.5 mL) mixed with DPPH radical solution (0.3 mL) was used as control. Mensor *et al*. [30] were used for scavenging activity percentage (%AA). The calculation formula is as follows equation (1)

$$\%AA = 100 - [(\frac{Abs_{sample-}\ Abs_{blank}}{Abs_{control}}) \times 100] \quad (1)$$

2.4.5 X-ray Diffractometry (XRD)

The AgNPs powders were identified using XRD (Philips X'Pert-MDP), and the target was Cu and K with a wavelength of 1.54060 Å. The generator was operated at 40kV with a 30 mA current. The scanning range was selected between 10.00 and 90.00, and the scan speed was 2.00 [31].

2.5 Statistical Analysis

The data were expressed as mean values ± standard deviation for each measurement and analyzed by means of the analysis of variance (One-Way ANOVA). A probability of $P<0.05$ indicates that the values are considered statistically significant.

3. Results and Discussion

3.1. Phytochemical Screening

Preliminary phytochemical testing revealed the active substances for the pharmacological activity evaluation of the sample weeds *(Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L.). The extracts possessed a variety of different secondary metabolites (Table 1).

Table 1. Phytochemical screening of Chromolaena odorata L. aqueous extract, *Amaranthus virdis* L., and *Cyperus Rotudus* L.

Class of Compounds	Aqueous Extract		
	***Chromolaena odorata* L.**	***Amaranthus virdis* L.**	***Cyperus Rotudus* L.**
Cardiac glycoside	+	+	-
Flavonoids	+	+	+
Saponins	-	+	-
Tannins	+	-	+
Terpenoids	+	+	-
Anthraquinones	-	-	-
Coumarin	+	+	+
Phlobatannins	-	-	-
Steroids	-	-	-
Alkaloids	-	-	-
Glycoside	-	-	-

Note: (+) Present, (-) Absent

Table 1 demonstrates the active compounds of *Chromolaena odorata* L leaf extracts: cardiac glycoside, flavonoids, tannins, terpenoids, and coumarin. The analysis results were consistent with the Kavitha et al. [33]. The analysis of ethanolic and aqueous extracts of *Chromolaena odorata* L. leaf and *Annona squamosa* seed showed

free radical scavenging, antimicrobial, and antimicrobials, which prevent infections, indicating phytochemical compounds and pharmacology actives. The aqueous extracts of *Chromolaena odorata* L. leaf were found to have secondary substances with antioxidant and therapeutic potential. The secondary substances analyzed were obtained from Amaranthus virdis L. leaf extracts containing cardiac glycoside, flavonoids, terpenoids, and coumarin. Saud *et al*. [34] proposed the properties of *Amaranthus virdis* L. extracts based on leaf and seed (solvent: 80% methanol). *Amaranthus virdis* L. extracts possessed active compounds such as cardiac glycoside, tannins, and flavonoids, which revealed an antioxidant activity of IC50 83.45 - 75.95 µg/mL and a minimal inhibitory concentration (MIC) of extracts ranging from 178 -645 µg/mL. The pharmacological and phytochemical profile of crude extracts of *Amaranthus spinosus* L. leaf was investigated. The results revealed the presence of carbohydrates, phenolic compounds, phytosterol, alkaloids, and flavonoids, indicating the pharmacological potential of the leaves [35]. Tannins (antiinflammatory and antimicrobial), saponins (antibacterial), flavonoids (treat heart disease), alkaloids (treat fever and headache), steroids (regulate hormonal system), phenolic compounds, proteins (help in the growth of the organism) and anthraquinones (used in the dye industry) were reported [36]. Therefore, *Amaranthus spinosus* L. is classified as a medicinal plant with a wide pharmacological application [37].

The phytochemical analysis results of *Cyperus Rotudus* L. leaf extracts revealed flavonoids, tannins, and coumarin. Eman et al. [38] studied the phytochemical, antimicrobial, and GC-MS analysis methanolic (85%) extract of Cyperus Rotudus, which showed tannins, carbohydrates, phytosterols, and alkaloids. This indicates that the plant is biologically active and can be used as antibacterial and antifungal medicine. Khalid et al. [39] described the extraction of dried *Cyperus Rotudus* L. with water and 70% ethanol. The aqueous extract's phytochemicals were alkaloids, flavonoids, phenols, phlorotannins, saponins, tannins, and terpenoids, which showed antibacterial and antifungal activity. Preliminary phytochemical analysis results in this study showed that some substances were linked to flavonoids and coumarin. Cardiac glycoside and terpenoids present in *Chromolaena odorata* L. and *Amaranthus virdis* L., and tannins found in *Chromolaena odorata* L. and *Cyperus Rotudus* L, demonstrated the pharmacological activity of the sample weeds with the property of synthesizing the silver nanoparticles based on green synthesis [9].

3.2 Chemical Constituents Characterization

GC-MS carried out the active compounds analysis of sample weed extracts (Chromolaena odorata L., Amaranthus virdis L. and Cyperus Rotudus L.). The phytochemical analysis results are shown in Tables 2-4.

Table 2. Compounds Identification of *Chromolaena odorata* L. extracts.

Phytochemical compound	R.Time	Area%	Formula	Pharmacological activity	Reference
Terpinen-4-ol	12.215	2.79	$C_{10}H_{18}O$	Antibacterial, Antioxidant Antibiofilm	Laísa *et al*, (2020)
1,6-anhydro-beta-D-Glucopyranose	21.056	1.84	$C_6H_{10}O_5$	NR	NR
2,2,4-Trimethyl-1,3-pentanediol di isobutyrate	25.112	3.26	$C_{16}H_{30}O_4$	NR	NR
n-Hexa-decanoic acid	39.715	5.42	$C_{16}H_{32}O_2$	Antioxidant, pesticide	Hema *et al*, (2011)
Hexa-decanoic acid, ethyl ester	40.735	8.74	$C_{18}H_{36}O_2$	Antioxidant, pesticide	
Phytol	44.109	8.19	$C_{20}H_{40}O$	Anticancer	
cis-Vaccenic acid	44.677	5.01	$C_{18}H_{34}O_2$	Bioactive compound	Okereke *et al*, (2017)

Note: NR (not reported)

The analysis revealed the compounds with pharmacological effects and unspecified compounds**.** The chemical analysis of *Chromolaena odorata* L. leaves extracts showed seven compounds. Terpinen-4-ol compound is responsible for antibacterial and antibiofilm properties, as was found by Laísa *et al.* [40], who described the inhibition study of *Staphylococcus aureus*. Terpinen-4-ol demonstrated a minimal inhibitory (MIC = 0.25% (v/v)) and minimal bactericidal concentrations (MBC= 0.5% (v/v). N-hexa-decanoic n-hexadecanoic acid, hexadecanoic acid, and ethyl ester were found as antioxidants and pesticides. Phytol from *Chromolaena odorata* L. leaves extracts were analyzed and found to have anticancer properties [41]. As a bioactive constituent, the compound cis-Vaccenic acid corresponded to Okereke *et al*. [42], which evaluated *Tithonia diversifolia* gray leaf extracts. Bioactive compounds of the extracts possessed anti-infective action and treatment of endemic diseases. The pharmacology potential of two compounds, 1,6-anhydro-beta-D-Glucopyranose and 2,2,4-Trimethyl-1,3-pentanediol di isobutyrate, could not be identified. l-(+)-Ascorbic acid 2,6-dihexadecanoate and eicosanoic acid, ethyl ester were found in *Amaranthus virdis* L. extracts as reported by Igwe and Okwunodulu [43]. A study analyzed the phytochemical profiles of *Phyllanthus amarus* leaf extracts by GC-MS and found bioactive compounds used for treatment and medicinal herbs. Oleic acid compound with allergenic, anti-alopecia, anti-androgenic, anti-inflammatory, and reduction of hypocholesterolemia properties was also found [41]. The analysis results of *Cyperus Rotudus* L. extracts revealed t (+)-2-bornanone (antibacterial) [44], terpinene-4-ol (antibacterial and antibiofilm) [40].

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The analysis results of *Cyperus Rotudus* L. extracts revealed t (+)-2-bornanone (antibacterial) [44], terpinene-4-ol (antibacterial and antibiofilm) [40]. Copaene is a tricyclic sesquiterpene found in the essential oils of medicinal plants. Hasan *et al*. [45] reported copaene as an antioxidant likely to possess an anticancer effect. Copaene can be used in functional foods for pharmacological purposes. The analysis results revealed four compounds without pharmacological properties (Cyclohexanol, 4-(1,1-dimethyl ethyl)-, acetate, cis-, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-, 2,4,6-Tris(1,1-dimethyl ethyl)-4-methylcyclohexa-2,5-dien-1-one and 2,2,4-Trimethyl-1,3-pentanediol di-isobutyrate).

3.3 Reducing Agent Analysis

For reducing agent qualification testing, both litmus paper and organic solvent testing methods were used, which revealed the identity of the properties suitable for use as a precursor of the biological synthesis of silver nanoparticles. The results of the experiment are shown in Table 5.

Table 3. Compounds Identification of *Amaranthus virdis* L. extracts.

Phytochemical compound	R.Time	Area%	Formula	Pharmacological activity	Reference
l-(+)-Ascorbic acid 2,6-dihexadecanoate	39.724	24.55	$C_{38}H_{68}O_8$	Antioxidant	Igwe & Okwunodulu, (2014)
Eicosanoic acid, ethyl ester	40.743	7.30	$C_{22}H_{44}O_2$	Antioxidant	
Oleic Acid	44.682	13.24	$C_{18}H_{34}O_2$	Allergenic, Anti-alopecia, Anti-androgenic, Antiinflammatory, Hypocholesterolemic	Hema *et al*, (2011)

Note: NR (not reported)

Table 4. Compounds Identification of *Cyperus Rotudus* L. extracts.

Phytochemical compound	R.Time	Area%	Formula	Pharmacological activity	Reference
(+)-2-Bornanone	11.262	2.86	$C_{10}H_{16}O$	Antibacterial	Somrithai & Narongrit (2019)
Terpinen-4-ol	12.216	4.91	$C_{10}H_{18}O$	Antibacterial, Antioxidant	Laísa *et al*. (2020)
Cyclohexanol, 4-(1,1-dimethylethyl)-, acetate, cis-	17.272	1.25	$C_{12}H_{22}O_2$	NR	NR
Copaene	17.535	1.85	$C_{15}H_{24}$	Antioxidant Anticancer	Hasan *et al*. (2014)
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	19.225	29.19	$C_4H_9NO_5$	NR	NR
2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	21.449	1.74	$C_{19}H_{32}O$	NR	NR
2,2,4-Trimethyl-1,3-pentanediol di isobutyrate	25.115	5.99	$C_{16}H_{30}O_4$	NR	NR
n-Hexa-decanoic acid	39.725	13.20	$C_{16}H_{32}O_2$	Antioxidant, pesticide	Hema *et al*. (2011)
Hexa-decanoic acid, ethyl ester	40.740	5.21	$C_{18}H_{36}O_2$	Antioxidant, pesticide	

Note: NR (not reported)

Table 5. Reducing agent testing of aqueous extract of *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L.

Method	Results		
	***Chromolaena odorata* L.**	***Amaranthus virdis* L.**	***Cyperus Rotudus* L.**
Carboxylic acid			
1. Litmus Test	-	-	-
2. H_2SO_4	-	-	-
3. NaOH	+	-	+
4.NaOH+$NaHCO_3$	-	+	-
5. $AgNO_3$	-	-	+
Amine			
1. Litmus Test	-	+	+
2. HCL	+	-	-

Note: (+) Present, (-) Absent

Functional group analysis of the sample weed extracts was done using the FTIR spectroscopy technique, which evaluated the composition of the action group with reducing properties. The middle infrared region of 550 - 4,000 cm^{-1} was used. The molecules light absorption resonance is expressed as a frequency relation or wave number. Each functional group represented a wavelength specific to the bond oscillation [46]. The weed extracts were evaluated the reducing moiety, as shown in Figure 2.

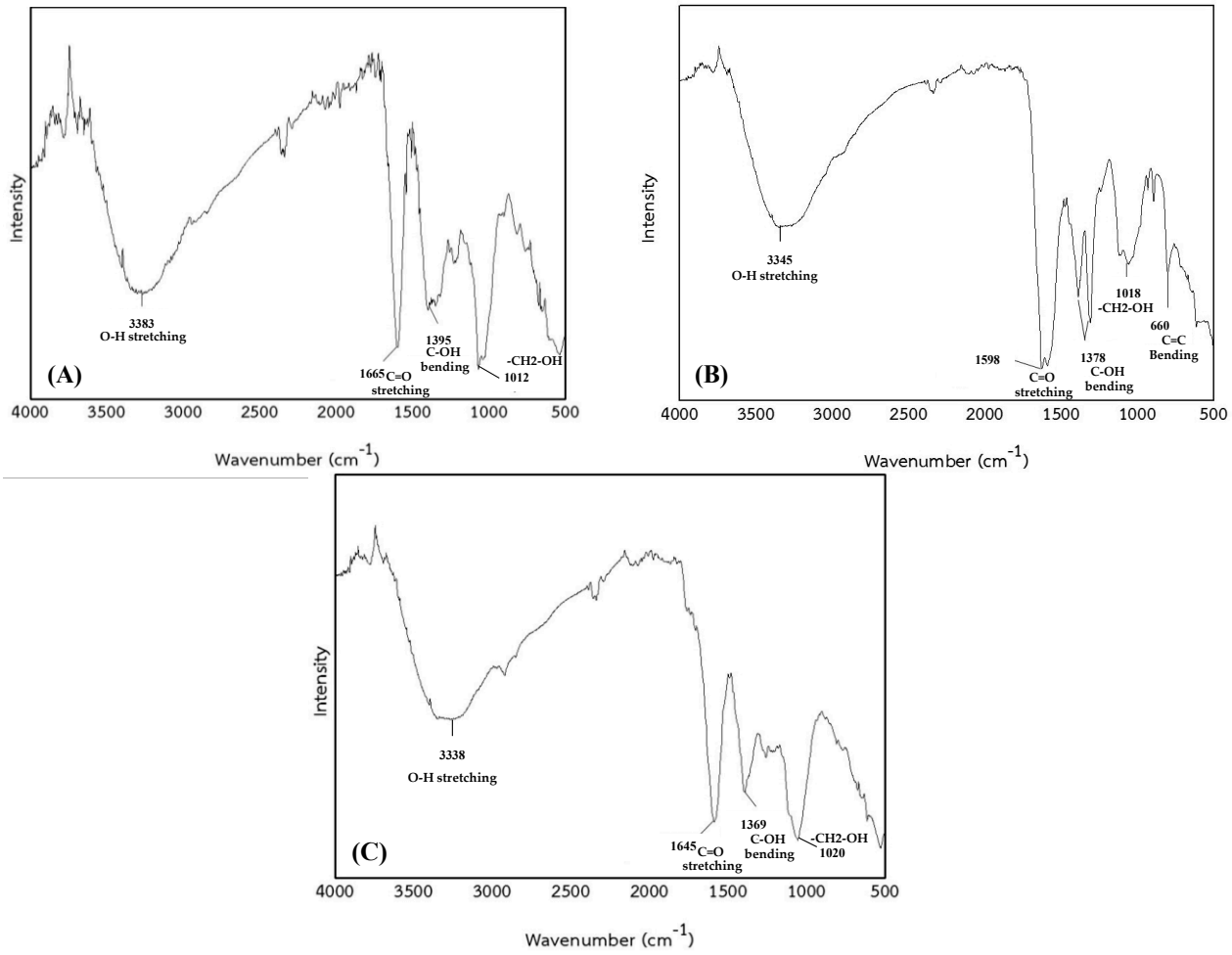

Figure 2. FTIR spectrum of crude extracts of (A) *Chromolaena odorata* L., (B) *Amaranthus virdis* L., and (C) *Cyperus Rotudus* L.

Table 6. Functional group of reducing agent testing of aqueous extracts of *Chromolaena odorata* L., *Amaranthus virdis* L. and *Cyperus Rotudus* L.

Extracts	Band range (standard) (cm^{-1})	Band range (experiment) (cm^{-1})	Assignments (interaction)	Possible compounds
Chromolaena odorata L.	1250-1000	1012	-CH_2-OH	carbohydrate
	1440-1339	1395	C-O-H (Bending)	phenol, alcohol
	1650-1550	1635	C=O (Stretching)	conjugated acid
	3400-3300	3385	O-H (Stretching)	Alcohols, Phenols
Amaranthus virdis L.	900-660	660	C=C (Bending)	alkene
	1250-1000	1018	-CH_2-OH	carbohydrate
	1440-1339	1378	C-O-H (Bending)	phenol, alcohol
	1650-1550	1598	C=O (Stretching)	conjugated acid
	3400-3300	3345	O-H (Stretching)	Alcohols, Phenols
Cyperus Rotudus L.	1250-1000	1020	-CH_2-OH	carbohydrate
	1440-1339	1369	C-O-H (Bending)	phenol, alcohol
	1650-1550	1645	C=O (Stretching)	conjugated acid
	3400-3300	3338	O-H (Stretching)	Alcohols, Phenols

Table 6 expresses the FTIR spectroscopy results of the aqueous extracts of *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L. showed the presence of the reducing agent. *Chromolaena odorata* L. extracts showed the vibration mode of -CH_2-OH groups of carbohydrate (1012 cm^{-1}) [57], C-O-H Bending (1395 cm^{-1}), C=O Stretching (1635 cm^{-1}), and O-H Stretching (3385 cm^{-1}) of alcohols or phenols [58]. The extracts of *Amaranthus virdis* L. leaves demonstrated the assignments of C=C Bending **(**660 cm^{-1}**),** -CH_2-OH groups of carbohydrate **(**1018 cm^{-1}**)** [57]**,** C-O-H Bending **(**1378 cm^{-1}**),** C=O Stretching **(**1598 cm^{-1}**)** and O-H Stretching (3345 cm^{-1}). The interaction and assignment of *Cyperus Rotudus* L. presented -CH_2-OH groups **(**1020 cm^{-1}**)** [57]**,** C-O-H Bending **(**1369 cm^{-1}**),** C=O Stretching **(**1645 cm^{-1}**)** and O-H Stretching (3338 cm^{-1}) [58].

The FTIR results correlated with the following literature: Man and Amorn [47] discussed the standard range of the interaction and assignment, which indicated the molecules uniqueness of the extract constituent compounds. Mythily and Devika [48] performed the extraction and pure isolation of *Fragaria xananassa* fruit, whereby the phytochemical, pure constituents, and antioxidant activity were evaluated. *Fragaria xananassa* fruit extracts contain aliphatic amines, indicating C-N s stretching vibration (1114.1919 cm^{-1}). Prince *et al*. [49] reported amines (1161 cm^{-1}, 1231 cm^{-1}) and alkene (721 cm^{-1}) from polyherbal formulations. The phenol, alcohol (1377 cm^{-1}), and conjugated acid (1686 cm^{-1}) were measured.

The phytochemical constituents and FTIR analysis of *Clitoria ternatea* leaf extracts were evaluated. The active compounds and bioactive constituents were Phenols (3389.57 cm^{-1}), Primary amines (1632.33 cm^{-1}), and Carboxylic acids (1057.61 cm^{-1}) [51]. This analysis revealed the properties of the compounds extracted from the sample weeds and the reducing agent of the active constituents infused in the extracts for the synthesis of silver nanoparticles using a green chemistry approach.

3.4 Inhibition Activity Percentage

Antioxidant agents suppress or reduce oxidative reactions, affecting the formation of free radicals that cause disease [52]. The antioxidant activity percentage (AA%) was evaluated by comparing the extracts of weed samples to ascorbic acid, which was inhibition of DPPH radical (Table 7). The weed sample extracts expressed AA% of 71.33% for *Chromolaena odorata* L., 18.36% for *Amaranthus virdis* L., and 21.74% for *Cyperus Rotudus* L. which, when compared to ascorbic acid (95.38%) had significant differences at $p<0.05$. The samples had a lower percentage of antioxidants than the standard. Si *et al*. [53] described the results of ethanolic extracts of *Cancrinia discoidea* to have anti-nociceptive, antiinflammatory, and antioxidant activities. Inhibition activity changes with the value of active constituents infused in extracts. The antioxidant potential of unripe and ripe *Citrus aurantifolia* was measured and compared to ascorbic acid. The juice concentration of *Citrus aurantifolia* increased, which affected the antioxidant activity percentage as 28.23% for unripe and 23.56% for ripe [54].

Table 7. Antioxidant activity percentage (AA%) of aqueous extracts of *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L.

Sample	% inhibition $_{Avg}$	S.D.	% RSD
Ascorbic acid	95.38	0.0002	0.0680
Chromolaena odorata L.	71.33	0.0007	0.2223
Amaranthus virdis L.	18.65	0.0005	0.1678
Cyperus Rotudus L.	21.74	0.0001	0.1508
F-test	1057553.494		
P-value	0.000[†]		

[†]Between groups within a column: One-Way ANOVA, $p < 0.05$

3.5 Silver nanoparticle Identification (XRD-Analysis)

Silver nanoparticles have the properties of anti-pathogenic or drug delivery [50] as synthesized by the green approach [13]. The silver nanoparticles identity was achieved through X-ray diffraction technique (XRD). Silver particles have the pattern of 2θ (planes for silver) at 38°(1 1 1), 45°(2 0 0), and 65°(2 2 0) [55]. Black sedimentary powders were achieved from the synthesis of silver nanoparticles by using sample weed extracts (*Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus* L.)

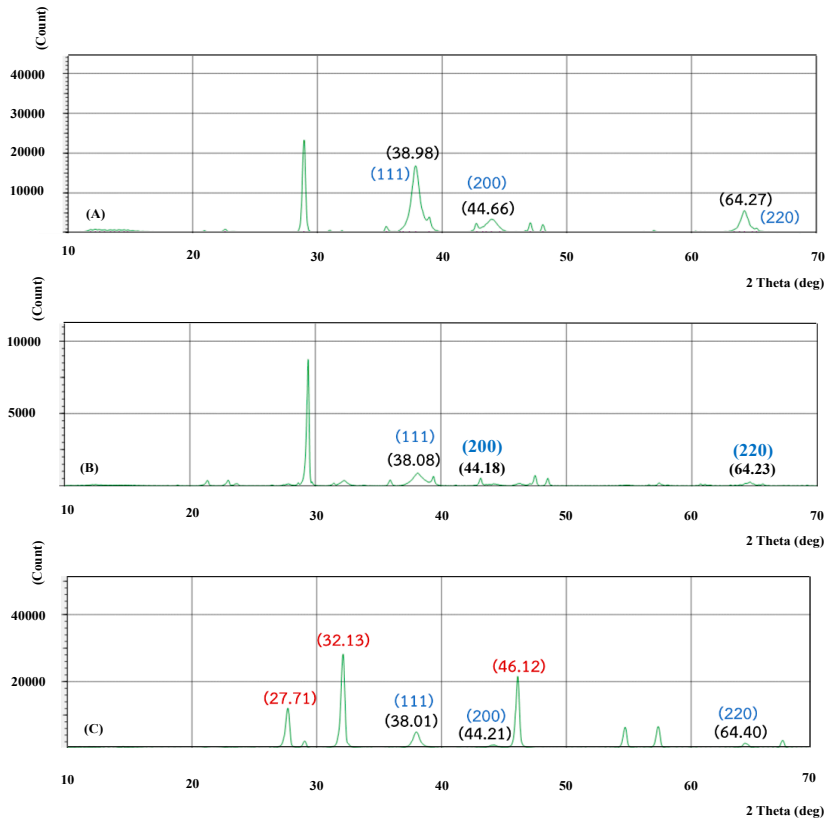

Figure 4. XRD patterns of AgNPs were obtained using (A) *Chromolaena odorata* L., (B) *Amaranthus virdis* L., and (C) *Cyperus Rotudus* L., reducing agents.

The product identity results are shown in Figure 4. The XRD patterns of silver nanoparticles synthesized from *Amaranthus virdis* L. extracts at 38.08°, 44.18°, and 64.23° were presented in planes (111), (200), and (220), respectively. Silver particle products from *Cyperus Rotudus* L. extracts expressed at 38.01°, 44.21°, and 64.40°, which were the planes at (111), (200), and (220), respectively. This corresponded to the XRD patterns of *Chromolaena odorata* L. at 39.98°(111), 44.66°(200), and 64.27° (220). Geetha *et al*. [59] discussed the synthesis process of silver nanoparticles by using the leaf extracts of *Cissus quadrangularis* L., which revealed the XRD-patterns of 2θ (1 1 1), (2 0 0), (2 2 0), and (311), that found the particle size of 11 nm. The silver nanoparticles synthesized from *Cyperus Rotudus* L. extracts demonstrated a high-intensity peak of 2θ at 27.71°, 32.13°, 46.12°. Picoli *et al*. [57] described the synthesized silver nanoparticles from *Fusarium oxysporum* extracts.

The XRD-patterns revealed the presence of three distinct peaks representing Bragg reflections of 27.9°, 32.3°, 46.3°, 55.0°, 57.6°, 67.6°, 74.6°, 76.9° and 85.7° relating to (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2), (4 0 0), (3 3 1), (4 2 0) and (4 2 2) planes, respectively were AgCl characteristic at, which were aggregates of silver particles. The results of the experiment confirmed the ability to silver with plant extracts that contain water as solvents. The nanoparticles are dispersed only in water. [19]. Green synthesized silver nanoparticles using *Cyperus rotundus* L. extract were determined by dynamic light scattering (DLS) with a Zeta sizer Nano ZS (Malvern, UK) instrument at 25 °C. [60]. This is a suggested concept used to analyze the size and morphology of synthesized particles. To determine the size of the synthesized material from this research.

4. Conclusions

Phytochemical extraction from three local weeds was obtained in Sakon Nakhon Province: *Chromolaena odorata* L., *Amaranthus virdis* L., and *Cyperus Rotudus*. The preliminary testing of chemical constituents found secondary metabolites which had pharmacological activity. Phytochemical screening was correlated with the results of the GC-MS profile of the sample weed extracts, which expressed bioactive

compounds. The pharmacological activity can evaluate the sample weed's potential for the synthesis of silver nanoparticles. The antioxidant potential was performed by inhibition percentage based on DPPH. The sample weed extracts demonstrated a lower inhibition percentage than ascorbic acid, with significant differences at $p<0.05$. Reducing agent determination was carried out with conventional method and instrument analysis, revealing the reducing compounds as phenol and carboxylic acid. XRD patterns of black powder from the synthesized silver nanoparticles confirmed the Ag identity. The results showed the effectiveness of the weeds for medicinal application, which was the production of AgNPs. Literature has discussed using green synthesis for the nano-sized synthesis of silver nanoparticles. The results could guide how local weeds can be used for medicine, pharmacological activity, and synthesis of metal particles.

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