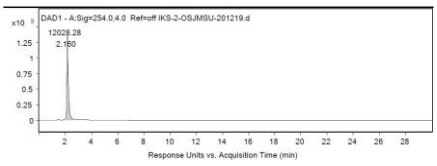
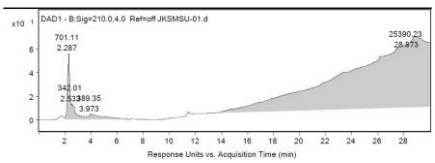
# Chapter 4

Phytochemical characterization of *Andrographis paniculata* ethanol and *Commiphora wightii* aqueous extracts




## 4.1 Introduction

**Figure 4.1: Morphology of *Andrographis paniculata* (Burm.f.) Nees.**

*Andrographis paniculata* (Burm.f.) Nees, a popular Indian medicinal herb from the family *Acanthaceae* is commonly known as "King of Bitters", traditionally as Kalmegh or Green Chirayta. It is a perennial herb, aboriginal to India and Srilanka and is distributed in different regions of Southeast Asia, China, America, West Indies and Christmas Island. In India, it is mainly found throughout the plains of the country and is cultivated in gardens especially in Bengal. It is known as Mahatikta in Sanskrit, Mahatita in Hindi, Kiryato in Gujarati and Kalmegh in Bengali. *A. paniculata*, the Kalmega of Ayurveda, is a bristling handsome herb that runs 60-70 cm in height and that holds eminently bitter taste in each and every part of the plant body (Figure 4.1). The plant with simple, opposite, lanceolate, glabrous, 2-12 cm long and 1-3 cm wide acute apex leaves that are dark green in color is festooned with small white flowers with rose-purple markings. It is one of the important herbs used in various Ayurvedic formulations, which were used to treat infections and diseases even before the advent of antibiotics. Traditional plant practice spectrum of this plant ranges from its usage to treat general ailments like inflammation, wounds, cuts, boils and skin diseases to disorders such as pyrexia, dysmenorrhea and leucorrhea and complicated diseases such as jaundice and gonorrhea. In the Ayurvedic medicinal system, tribals of Tamil Nadu, India use this herb for the treatment of malaria (Okhuarobo et al., 2014).

*Commiphora wightii* (Arn.) Bhandari a shrub from the *Burseraceae* family, is a highly valuable medicinal plant from Indian traditional systems of medicine and also holds a nice status in the modern drug system. Distinctive of *C. wightii* is the earthy aromatic fragrance with mossy and skunk-like notes. The plant has around 4 m height and has numerous colored, crooked and spirally ascending branches ending in sharp spines (Figure 4.2). The green barks of the plant are coated by shiny, ash to yellowish-white papery cover, which peels off in rolls. It has leaves that are deciduous, sessile and simple or trifoliate with 1-5 cm long ovate leaflets which are leathery

**Figure 4.2: Morphology of *Commiphora wightii* (Arn.) Bhandari**

and shiny green on top, greyish from bottom and have irregularly serrated edges. To embellish the plant further, small brownish-red sessile single or fascicles of flowers and berry-like red drupe ripe fruits of 7-8 mm diameter are present. It is disturbed in arid and semi-arid zones of northern Africa and tropical Asia. In India, it grows wild in Rajasthan, Gujarat (mostly Aravallis) and to a small extent in adjoining Madhya Pradesh and Maharastra (Ved et al., 2015). It derives its generic name from the Greek words "Phora" and "Kommis" which means gum bearer. Well-known as "Indian Bdellium" in English, Mahisaksha, Guggulu, Amish, Palanksha and Pur in Sanskrit, it is popular as Guggul in most of the Indian languages (Vani et al., 2016). The oleo gum resin, "Gum Guggul" or "Indian Myrrh" is an exudate from this plant that is useful in the treatment of arthritis, gout, inflammation, lipid disorders, nodulocystic acne and obesity (Jain and Nadgauda, 2013). This myrrh when brunt produces a pronounced calming effect on mind and is an important smudging incense in India and Pakistan. *Commiphora* genus plants are widely used to treat malaria by the Maasai community in Kenya and Tanzania traditionally (Koch et al., 2005; Kweyamba et al., 2019).

The *A. paniculata* EtOH extract and the *C. wightii* AQ extract were found to exhibit significant inhibition of recombinant *P. falciparum* specific PMs, mPM I and mPM II. Thus the extracts were explored for their phytochemical composition with the aim to find out the compounds with PM inhibition property and thus leading to the PM inhibitors of natural origin.

## 4.2 Material and methods

### 4.2.1 Qualitative analysis of phytochemical constituents

The major phytochemical constituents in the extracts were screened using standard procedures (Auwal et al., 2014; Bankole et al., 2016; Banu and Cathrine, 2015; Sheel et al., 2014; Vimalkumar et al., 2014). Inferences were drawn based on the results from the experiments performed in triplicate.

### 4.2.2 Quantitative analysis of phytochemical constituents

For estimating the amount of detected phytochemicals in the extracts standard procedures with few modifications were followed. All the estimations were done in triplicate.

#### *4.2.2.1 Total alkaloid content determination*

For this gravimetric method described by Bankole et al. (2016) was used. Five grams of extract was dissolved in 200 ml of 10% acetic acid in ethanol and the solution was stood for 4 h. The filtrate obtained from the solution was concentrated on a water bath to 1/4$^{th}$ of the original volume. The alkaloids were precipitated out completely with drop-wise addition of concentrated ammonium hydroxide. The precipitates were collected and washed with dilute ammonium hydroxide. The residue obtained after filtration was dried completely into a constant weight and measured. The content of the total alkaloids was expressed as µg of alkaloids present per mg of extract (µg/mg).

#### *4.2.2.2 Total amino acids content determination*

This was done according to the method described by Wang (n.d.). One milligram of extract was dissolved in 1 ml of distilled water and vortexed. To this, 0.5 ml of 1% ninhydrin was added and mixed. The reaction was kept in a boiling water bath (80-100°C) for 20 min. The reaction was cooled using a cold water bath. The intensity of the purple color developed was recorded at 570 nm against a blank where 1 ml of the extract sample was replaced by distilled water. The above procedure was followed to plot a standard curve using L-arginine solutions at different concentrations (100-1000 µg/ml). The total amount of amino acids was calculated as µg of L-arginine equivalents present per mg of extract (µg/mg).

#### *4.2.2.3 Total carbohydrate content determination*

Total carbohydrates in extract were quantified using the phenol-sulfuric acid method. For this, 2 ml of distilled water was added to 10 mg of extract and the mixture was boiled for 30 min. After mixing briefly the solution was centrifuged. The supernatant was collected for analysis. The standard solutions of glucose were prepared to range from 0.02-0.1 µg/ml of glucose. To 1 ml of the standard or extract solution, 1 ml of 5% phenol and 5 ml of sulfuric acid were added. The reagents were mixed carefully and stood for 10 min. The reactions were cooled in a water bath at 25-30°C for 15 min. The reactions were read at 490 nm against reaction blank where distilled water was substituted for the sample solution.

The amount of carbohydrates in the extract was then calculated in terms of glucose equivalents (µg/mg).

#### *4.2.2.4 Total flavanoid content determination*

This was done using the aluminum chloride method. Stock solution of plant extract was prepared by dissolving 10 mg of extract in 1 ml of methanol. For the assay, to 0.5 ml of the stock solution 1.5 ml of methanol was added. This was followed by the addition of 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. All the reagents were mixed well and the reactions were incubated for 30 min. The amount of total flavanoids was estimated in terms of µg of quercetin equivalents present per mg of extract (µg/mg). For this, a standard curve was plotted using different concentrations of quercetin solutions ranging from 6.5-100 µg/ml, prepared in methanol. After incubation, the absorbance of the reactions was read at 415 nm against a reaction kept as a blank, where the test or standard solution was replaced by methanol.

#### *4.2.2.4 Total phenolic content determination*

This was done using the spectrophotometric method using Folin-Ciocalteau (F-C) reagent. Briefly, 1 mg of extract was dissolved in 1 ml of methanol. Then 0.2 ml of the extract solution was mixed with 1.5 ml of 10% aqueous F-C reagent. The reaction was incubated in dark for 5 min. Finally, 1.5 ml of 5% $Na_2CO_3$ was added to the solution and the contents were mixed well. Again the reaction was incubated in dark for 2 h. The absorbance was measured at 760 nm. The concentration of the total phenolics was estimated as µg of gallic acid equivalents per mg of extract (µg/mg) by using a gallic acid standard curve. For the standard curve, the above procedure was followed to read different concentrations of gallic acid solutions ranging from 50-200 µg/ml, prepared in methanol.

#### *4.2.2.5 Total saponin content determination*

For this, the gravimetric method described by Bankole et al. (2016) was used. Five grams of plant extract was dispersed in 50 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with intermittent stirring at about 55°C. The suspension was filtered and the residue obtained was re-extracted with another 50 ml of 20% ethanol. The filtrates were pooled and reduced to 40 ml by heating over a water bath at about 90°C. Then the concentrate was shaken vigorously with 20 ml of diethyl ether in a 250 ml separating funnel

for about 10 min. The aqueous layer was collected. The purification process was repeated with the collected layer using 60 ml of n-butanol. The saponin carrying n-butanol phase was collected and washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was evaporated using a water bath at about 90°C. The sample was dried in oven into a constant weight and measured. The total saponins were expressed as µg of saponins present per mg of extract (µg/mg).

#### *4.2.2.6 Total steroid content determination*

The spectrophotometric assay using Liberman-Burchard (L-B) reagent was used for this. Briefly, 2 g of extract was dissolved in 5 ml of chloroform. To this 2 ml of L-B reagent (0.5ml of concentrated sulfuric acid in 10 ml of acetic anhydride) was added and mixed well. The reactions were covered with black paper and kept in dark for 15 min. The green-colored complex formed was read at 640 nm. A reaction where sample volume was replaced with chloroform was kept as a blank during the procedure. The amount of steroids was determined in terms of cholesterol equivalents (µg/mg) using cholesterol as a standard. For this, the above procedure was followed with cholesterol solutions in chloroform at various concentrations ranging from 250-2500 µg/ml to plot a cholesterol standard curve. Dilutions were done in chloroform, keeping the dilution factor constant for standard and test samples to avoid incorrect readings due to solvent variability.

### 4.2.3 LC-MS-QTOF analysis

The separation and mass analysis of the components of the antiplasmodial extracts showing PM inhibition property were performed with LC system (Agilent Technologies) using a C-18 column equipped with a photodiode array detector, coupled with a Q-TOF mass spectrometer and 6200 series TOF/6500 series Q-TOF B.08.00 (B8058.0) data processing software. For both the *A. paniculata* EtOH extract and *C. wightii* AQ extract, a gradient of water:methanol was used as mobile phase, at a temperature of 25°C at a flow rate of 0.4 ml/min, where 90% water and 10% methanol started the gradient. The detection wavelength used for the *A. paniculata* EtOH extract was from 190-600 nm while for the *C. wightii* AQ extract it was kept from 190-400 nm. The mass spectrometry analysis for both the extracts was performed in positive mode with the mass scan over the range of 100-1500 m/z. The fragmentation pattern and the base peaks in the mass spectra generated were

matched against the public databases such as NIST and PubChem as well as literature available was referred for identification of compounds.

## 4.3 Results and discussion

### 4.3.1 Qualitative and quantitative analysis of phytochemical constituents

Phytochemical analysis of the plant extracts exhibiting PM inhibition property was done to reveal the major principles present in them (Table 4.1). Qualitative analysis of the extracts showed the presence of alkaloids, carbohydrates, phenolics in both the tested extracts. Proteins, steroids and terpenoids were detected only in the *A. paniculata* EtOH extract and saponins and amino acids were found only in the *C. wightii* AQ extract. The phytochemicals suggested to be present in the extracts were then quantified (Table 4.2). The *A. paniculata* EtOH extract was found to be rich in terpenoids (480.87 ± 57.80 μg/mg) and contained a substantial amount of steroids (83.80 ± 23.70 μg/mg). Terpenoids have been reported earlier to account for a large proportion of phytochemical components of this plant (Okhuarobo et al., 2014). In the *C. wightii* AQ extract free amino acids (318.71 ± 25.44 μg/mg) and saponins (434.42 ± 49.22 μg/mg) were found in abundance. Similarly, the AQ fraction from *C. mukul* showed the presence of various amino acids (Hanus et al., 2005). Saponin glycosides richness in *C. mukul* documented before can explain the abundance of saponins found in the *C. wightii* AQ extract (Yarnell, 2007).

Saponins are well known for their hemolytic property (Sotheeswaran, 1988). It is interesting to mention that in spite of saponin abundance in the *C. wightii* AQ extract, it was found to be harmless to erythrocytes as mentioned in chapter 2. The $HC_{50}$ of the extract was found to be > 500 μg/ml which is a concentration well above than that required for this extract to exhibit antiplasmodial effect or other therapeutic effects in general. Similarly, lack of hemolytic potency has been reported for boswellic acids, which are the main sapogenins of the gum resin of a plant from the *Burseraceae* family, *Boswellia serrata* (Vo et al., 2017) also known as Salai guggul in Sanskrit. This indicates the existence of boswellic acids or similar sapogenins as the main constituent aglycones of *C. wightii* saponins. In addition, our observation corroborates the statement that various structural aspects, such as the nature of the aglycone backbone, the complexity of sugar moieties, and the number, length and position of sugar side chains, may affect hemolytic activity (Vo et al., 2017).

**Table 4.1: Qualitative phytochemical screening of *A. paniculata* EtOH extract and *C. wightii* AQ extract.**

| Phytochemical constituents | Tests | *A. paniculata* EtOH | *C. wightii* AQ |
|---|---|---|---|
| Alkaloids | Dragendroff's test | - | ± |
| | Mayer's test | ± | ± |
| | Wagner's test | - | ± |
| Carbohydrates | Molish test | ± | ± |
| | Benedict's test | ± | ± |
| | Fehling's test | ±± | ± |
| Flavanoids | Ammonia test | - | - |
| | Lead acetate test | - | - |
| | Shinoda's test | - | - |
| Phenolics | Dilute iodine solution | - | ± |
| | Ferric chloride test | ± | - |
| | Lead acetate test | ± | ±± |
| Proteins | Biruet test | ± | - |
| Saponins | Froth test | - | ± |
| Steroids | Liebermann-Burchard | - | - |
| | Salkowaski test | ± | - |
| Terpenoids | Liebermann-Burchard | ± | - |
| Amino acids | Ninhydrin test | - | ±± |
| Anthraquinone | Borntrager's test | - | - |
| Phlobatannins | HCl test | - | - |

Legend: (-) absent, (±) low or reduced (±±) Abundant
Data summarized from observations recorded in triplicate

**Table 4.2: Quantitative phytochemical screening of *A. paniculata* EtOH extract and *C. wightii* AQ extract.**

| Phytochemical constituents | *A. paniculata* EtOH (µg/mg) | *C. wightii* AQ (µg/mg) |
|---|---|---|
| Alkaloids | 52.51 ± 12.77 | 127.15 ± 31.53 |
| Amino acids | 0.00 ± 0.00 | 318.71 ± 25.44 |
| Carbohydrates | 0.17 ± 0.01 | 23.70 ± 3.34 |
| Phenolics | 11.79 ± 0.04 | 12.08 ± 1.76 |
| Proteins | 0.45 ± 0.06 | 0.00 ± 0.00 |
| Saponins | 0.00 ± 0.00 | 434.42 ± 49.22 |
| Steroids | 83.80 ± 23.70 | 0.00 ± 0.00 |
| Terpenoids | 480.87 ± 57.80 | 0.00 ± 0.00 |

Data is presented as mean ± standard deviation from experiments done in triplicates

The amount of alkaloids were found to be higher in the *C. wightii* AQ extract (127.15 ± 31.53 µg/mg) than in the *A. paniculata* EtOH extract (52.51 ± 12.77 µg/mg), the levels of

carbohydrates were high in the case of the *C. wightii* AQ extract (23.7 ± 3.34 µg/mg) while they were very low in the case of the *A. paniculata* EtOH extract (0.17 ± 0.01 µg/mg) and there was no significant difference in the levels of phenolics found from both the extracts. The high levels of carbohydrates detected in the *C. wightii* AQ extract can be in part explained by the possible abundance of saponin glycosides in the extract as also reported from *C. mukul* (Yarnell, 2007). Although the *A. paniculata* EtOH extract was detected positive for proteins, their levels were found to be very low (0.45 ± 0.06 µg/mg) after quantification.

### 4.3.2 LC-MS-QTOF analysis

LC of the *A. paniculata* EtOH extract resulted in separation of phytoconstituents which had retention time (RT) ranging from 1.144-8.989 min (Figure 4.3A). The compounds were then analyzed by MS-QTOF (Table 4.3). Analysis of compounds with RT from 1.841-2.222 min and RT from 2.272-2.537 min suggested the presence of two labdane diterpenoids, andrographolide and 14-deoxy-11, 12-didehydroandrographolide in the extract. Terpenoids constituting the major portion of the *A. paniculata* EtOH extract as determined from phytochemical analysis, suggests that that andrographolide and 14-deoxy-11,12-didehydroandrographolide are two principle compounds of the extract. This finding is in support of the document stating these two as the most abundant diterpenoids in *A. paniculata* (Lin et al., 2019). Andrographolide has been earlier reported to exhibit antiplasmodial activity by Mishra et al. (2011). Additionally *in silico* studies have suggested andrographolide as a potentially active inhibitor of PM I, II and IV (Megantara et al., 2015). Altogether this suggests andrographolide as an antiplasmodial agent from *A. paniculata* that possibly acts by means of inhibiting the *P. falciparum's* PMs. Another remarkable result from the mass spectrum analysis of this extract was the presence of different classes of phospholipids namely phosphatidylcholines(PCs), phosphatidylethanolamines, phosphatidylserines and oleoyl lysophosphatidylcholines, which were profuse as indicated by their detection at RT values which varied in the large range of 1.144-8.989 min. Among all the phospholipids identified, most of the lipids belonged to PC class, possibly because they are the major phospholipid class present in the cell membrane of plants. The over-abundance of phospholipids found in the *A. paniculata* EtOH extract certainly is information on the constitution of the plant or if their profusion is temporary, it is a condition that needs clarification. One explanation to the latter case can be

provided by the unexpected transient increase of PCs at an early stage of phosphate starvation (Jouhet et al., 2003).

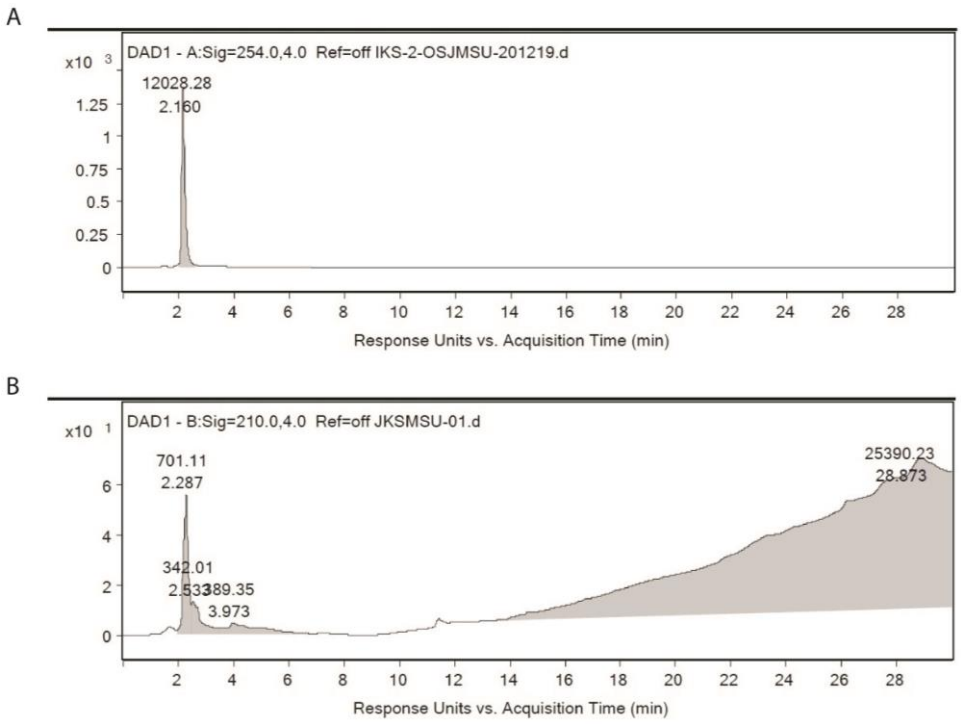



**Figure 4.3: LC chromatogram plant extracts.**

(A) Chromatogram of *A. paniculata* EtOH extract at wavelength from 190-600 nm. Major peak was detected at 2.160 min.
(B) Chromatogram of *C. wightii* AQ extract at wavelength from 190-400 nm. Major peaks were detected at RT 2.287 min and 28.873 min.

Separation of the *C. wightii* AQ extract constituents was achieved by LC (Figure 4.3B). The analysis of the eluent by MS-QTOF showed some major fragments that assisted in the identification of the compounds (Table 4.4). The mass spectrum for the compounds with RT from 1.274-7.444 min showed the presence of valine. Although some nitrogen-containing fragments similar to that of valine were present in the same zone, they could not be identified. The presence of high levels of free amino acids in the *C. wightii* AQ extract as determined from phytochemical analysis suggests that this fragmentation pattern belonged to other amino acids that coeluted. From the analysis of the compounds with RT from 14.742-15.057 min, a compound/fragment with molecular formula $C_{20}H_{30}O_{13}$ was detected and it is worth mentioning that this compound/fragment produced the most abundant ions, which implicates its abundance in the extract as well. Although NMR studies are required to elucidate its structure, it may be scroside D or a compound

**Table 4.3: Major fragments produced and components identified from *A. paniculata* EtOH extract by LC-MS-QTOF analysis.**

| RT (min) | m/z (Da) | Ion type | Compound monoisotropic mass | Formula | Putative identified compound |
|---|---|---|---|---|---|
| 1.144-1.442 | 199.0832 | [M+H]+ | 198.0753 | $C_7H_{10}N_4O_3$ | |
| | 215.078 | [M+Na]+ | 192.0881 | $C_8H_{18}NS_2$ | 1,3,5-dithiazinium-2,4,5,5,6- pentamethyldihydro- |
| | 229.0939 | [M+Na]+ | 206.1037 | $C_9H_{20}NS_2$ | 1,1-bis-methylsulfanylmethyl-piperidinium |
| | 783.5769 | [M+H]+ | 782.5697 | $C_{48}H_{78}O_8$ | 2,3-bis(4-hydroxy3-methoxybenzyl)butane-1,4-diylditetradecanoate, Fragment from $C_{46}H_{78}NO_8P$ |
| | 804.5552 | [M+H]+ | 803.5465 | $C_{46}H_{78}NO_8P$ | PC(38:7): PC(18:2/20:5) |
| | 806.5702 | [M+H]+ | 805.5622 | $C_{46}H_{80}NO_8P$ | PC(38:6) |
| | 806.5702 | [M+H]+ | 807.5778 | $C_{46}H_{82}NO_8P$ | PC(38:5) |
| | 808.5869 | [M+H]+ | 807.5778 | $C_{46}H_{82}NO_8P$ | PE (41:5) |
| 1.525-1.708 | 104.1086 | [M+H]+ | 103.0997 | $C_5H_{13}NO$ | Dimethylamino-1-propanol |
| | 758.5726 | [M+H]+ | 757.5622 | $C_{42}H_{80}NO_8P$ | PC (34:2): PC (16:0/18:2) |
| | 805.5581 | [M+H]+ | 805.5587 | $C_{60}H_{71}N$ | |
| | 806.5705 | [M+H]+ | 805.5622 | $C_{46}H_{80}NO_8P$ | PC(38:6): PC(16:0/22:6) |
| | 808.5872 | [M+H]+ | 807.5778 | $C_{46}H_{82}NO_8P$ | PC(38:5): PC (18:1/20:4) |
| 1.841-2.222 | 315.1976 | [M+H]+ | 314.1882 | $C_{20}H_{26}O_3$ | Fragment from 14-deoxy-11,12-didehydroandrographolide ($C_{20}H_{28}O_4$) |
| | 315.1976 | [M+H]+ | 332.1988 | $C_{20}H_{28}O_4$ | Fragment from andrographolide ($C_{20}H_{30}O_5$) |
| | 758.576 | [M+H]+ | 757.5622 | $C_{42}H_{80}NO_8P$ | PC (34:2): PC (16:0/18:2) |
| 2.272-2.537 | 315.1976 | [M+H]+ | 314.1882 | $C_{20}H_{26}O_3$ | Fragment from 14-deoxy-11,12-didehydroandrographolide ($C_{20}H_{28}O_4$) |
| | 315.1976 | [M+H]+ | 332.1988 | $C_{20}H_{28}O_4$ | Fragment from andrographolide ($C_{20}H_{30}O_5$) |
| | 780.5553 | [M+H]+ | 779.5465 | $C_{44}H_{78}NO_8P$ | PC (36:5): PC(18:2/18:3) |
| | 780.5554 | [M+H]+ | 779.5489 | $C_{51}H_{73}NO_5$ | |
| | 781.5811 | [M+H]+ | 781.5622 | $C_{44}H_{78}NO_8P$ | PC (36:4): PC(18:1/18:3) |
| | 804.5563 | [M+H]+ | 803.5465 | $C_{46}H_{78}NO_8P$ | PC(38:7): PC(18:2/20:5) |
| | 808.589 | [M+H]+ | 807.5778 | $C_{46}H_{82}NO_8P$ | PC(38:5): PC(18:0/20:5) |
| 2.603-3.267 | 780.5553 | [M+H]+ | 779.5465 | $C_{44}H_{78}NO_8P$ | PC (36:5): PC(18:2/18:3) |
| | 780.5554 | [M+H]+ | 779.5489 | $C_{51}H_{73}NO_5$ | |
| | 784.5811 | [M+H]+ | 781.5622 | $C_{44}H_{80}NO_8P$ | PC(36:4): PC(18:1/18:3) |
| | 806.5727 | [M+H]+ | 805.5622 | $C_{46}H_{80}NO_8P$ | PC(38:6): PC (20:5/18:1) |
| | 780.5553 | [M+H]+ | 779.5465 | $C_{44}H_{78}NO_8P$ | PC (36:5): PC(18:2/18:3) |
| | 809.5919 | [M+H]+ | 809.5935 | $C_{46}H_{84}NO_8P$ | PC(38:4): PC (18:1/20:3) |
| | 780.5553 | [M+H]+ | 779.5465 | $C_{44}H_{78}NO_8P$ | PC (36:5): PC(18:2/18:3) |
| | 804.5553 | [M+H]+ | 804.5543 | $C_{46}H_{78}NO_8P$ | PC(38:7) |
| | 875.6528 | [M+H]+ | 874.6537 | $C_{48}H_{93}NO_{10}P$ | PS (42:0) |
| | 875.6528 | [M+H]+ | 875.6404 | $C_{51}H_{90}NO_8P$ | PC (43:6) |
| | 118.089 | [M+H]+ | 117.079 | $C_5H_{11}NO_2$ | Valine |
| 3.416-4.942 | 118.0875 | [M+H]+ | 117.079 | $C_5H_{11}NO_2$ | Valine |
| | 760.5816 | [M+H]+ | 759.5743 | $C_{56}H_{73}N$ | |
| | 784.5833 | [M+H]+ | 783.5778 | $C_{44}H_{82}NO_8P$ | PC(36:3): PC (16:0/20:3) |
| | 804.5554 | [M+H]+ | 803.5465 | $C_{46}H_{78}NO_8P$ | PC(38:7) |
| 5.091-6.949 | 118.0874 | [M+H]+ | 117.079 | $C_5H_{11}NO_2$ | Valine |
| | 784.5836 | [M+H]+ | 783.5778 | $C_{44}H_{82}NO_8P$ | PC(36:3): PC (16:0/20:3), OLPC |
| | 808.5881 | [M+H]+ | 808.5856 | $C_{46}H_{82}NO_8P$ | PC(38:5): PC (20:5/18:0) |
| 7.065-8.989 | 118.0874 | [M+H]+ | 117.079 | $C_5H_{11}NO_2$ | Valine |
| | 102.1288 | [M+H]+ | 101.1204 | $C_6H_{15}N$ | |
| | 209.0944 | [M+H]+ | 208.0882 | $C_7H_{16}N_2O_3S$ | 2-amino-2-ethyl-4-(methylsulfonimidoyl)butanoic acid |
| | 209.0944 | [M+H]+ | 209.0943 | $C_8H_{18}O_4P$ | Diethyl(2-methylallyl)phosphate |
| | 780.5541 | [M+H]+ | 779.5465 | $C_{44}H_{78}NO_8P$ | PC (36:5): PC (16:0/20:5), PC(16:1/20:4) |
| | 782.5725 | [M+H]+ | 782.57 | $C_{44}H_{81}NO_8P$ | l,2-bis[2,4-(E,E)-octadecadienoyl]-sn-glycero-3-phosphocholine , PC(36:4) |

PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, OLPC: oleoyl lysophosphatidylcholine

**Table 4.4: Major fragments produced and components identified from *C. wightii* AQ extract by LC-MS-QTOF analysis.**

| RT (min) | m/z (Da) | Ion type | Compound monoisotropic mass | Formula | Putative identified compound |
|---|---|---|---|---|---|
| 1.274-7.444 | 104.1072 | [M+H]+ | 103.0997 | $C_5H_{13}NO$ | |
| | 140.0684 | [M+Na]+ | 117.079 | $C_5H_{11}NO_2$ | Valine |
| | 146.1176 | [M+H]+ | 145.1103 | $C_7H_{15}NO_2$ | |
| | 175.1192 | [M+$NH_4$]+ | 157.0851 | $C_6H_{11}N_3O_2$ | |
| | 203.0531 | [M+Na]+ | 180.0634 | $C_6H_{12}O_6$ | Glucose, myo inositol |
| | 215.0531 | [M+Na]+ | 192.0634 | $C_7H_{12}O_6$ | Quinic acid |
| | 224.1281 | [M+H]+ | 223.1208 | $C_{12}H_{17}NO_3$ | Anhalidine, Anhalonidine |
| 10.662-15.588 | 457.1319 | [M+Na]+ | 434.1424 | $C_{18}H_{26}O_{12}$ | Sorbitol hexaacetate |
| | 501.1584 | [M+Na]+ | 478.1686 | $C_{20}H_{30}NO_{13}$ | Scroside D |
| | 501.1585 | [M+Na]+ | 478.1686 | $C_{20}H_{30}NO_{13}$ | |
| | 502.1615 | [M+Na]+ | 478.1686 | $C_{20}H_{30}NO_{13}$ | |
| | 517.132 | [M+K]+ | 478.1686 | $C_{20}H_{30}NO_{13}$ | |
| 19.071-25.373 | 261.1107 | [M+H]+ | 260.1035 | $C_{13}H_{14}N_3O_3$ | |
| 25.456-27.463 | 163.0762 | [M+H]+ | 162.0681 | $C_{10}H_{10}O_2$ | Safrole, Methyl cinnamate |
| | 301.1423 | [M+H]+ | 300.1348 | $C_{16}H_{18}N_3O_3$ | |
| | 365.1376 | [M+H]+ | 364.1297 | $C_{20}H_{18}N_3O_4$ | |
| | 366.1411 | [M+H]+ | 364.1297 | $C_{20}H_{18}N_3O_4$ | |
| | 367.1433 | [M+H]+ | 364.1297 | $C_{20}H_{18}N_3O_4$ | |
| 27.612-29.884 | 297.2438 | [M+Na]+ | 279.2106 | $C_{13}H_{31}N_2O_2S$ | |
| | 365.137 | [M+H]+ | 364.1297 | $C_{20}H_{18}N_3O_4$ | |
| | 782.571 | [M+Na]+ | 759.5815 | $C_{35}H_{77}N_{13}OS_2$ | Saponin derivatives |
| | 786.6029 | [M+H]+ | 785.5963 | $C_{35}H_{69}N_{20}O$ | |
| | 810.6032 | [M+H]+ | 809.5950 | $C_{35}H_{67}N_{23}$ | |
| | 811.6064 | [M+Na]+ | 788.6198 | $C_{35}H_{72}N_{20}O$ | |
| | 812.6146 | [M+Na]+ | 788.6198 | $C_{35}H_{72}N_{20}O$ | |
| | 834.6015 | [M+K]+ | 795.6409 | $C_{39}H_{85}N_7O_9$ | |

similar to it. Scroside D, a phenylethanoid glycoside with powerful antioxidant activity is found in the plant *Stachytarpheta cayennensis* (Wang et al., 2004). Phenylethanoid glycosides are characteristic constituents of *Stachytarpheta cayennensis* which is used ethnomedically to treat the symptoms of malaria (Froelich et al., 2008). This raises the possibility of $C_{20}H_{30}O_{13}$ as an antiplasmodial principle from *C. wightii*. In addition, Scroside D or Scroside D like compound presence may also contribute to the antioxidant activity of *C. wightii*, along with the established antioxidants from this plant, Z-guggulsterones and E-guggulsterones (Jaiswal et al., 2016). Although the compounds that exhibit protease inhibition have not been identified until now from *C. wightii*, several classes of compounds with such action have been reported from many species of the *Commiphora* genus (Bakari et al., 2012). The role of the abundant compound scroside D or a similar compound as PM inhibitor can be found after further analysis.

Analysis of the compounds with RT from 27.612-29.884 min showed the presence of nitrogen and oxygen hydrocarbon compounds/fragments typically containing 35-39

carbon atoms. Two compounds/fragments with RT in this range viz., $C_{13}H_{31}N_2O_2S$ and $C_{35}H_{77}N_{13}OS_2$ also had the element sulfur. The chromatogram and the abundance of the molecular/parent ions produced by them suggest their presence in high levels in the AQ extract of *C. wightii*. Saponins were found in abundance in the *C. wightii* AQ extract during phytochemical analysis. Besides, similar fragments were generated by saponins from the medicinal plant *Vernonia amygdalina* Del (Wang et al., 2018). This strongly suggests that these molecular/parent ions are derivatives of compounds belonging to the class saponins. Studies ahead can reveal the identity of these heavy compounds from *C. wightii*.

In conclusion, the study revealed the compounds in the selected plant extracts which may have antiplasmodial and/or antiPM activity along with the other compounds which do not exhibit these activities but are potentially useful. The abundant compounds namely andrographolide from *A. paniculata* and scroside D or scroside D like compound from *C. wightii* are proposed as compounds with PM inhibitory activity. The findings might be useful in the development of PM inhibitors of natural origin. Implementation of bioinformatic tools may assist in identifying the compounds with putative PM inhibition activity from the plants and may also expose their mechanism of inhibition.