



## Phytochemical, thin layer chromatographic and IR spectroscopic studies on *Andrographis paniculata* of Kamrup of Assam, India

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### Abstract

*Kamrup district of Assam has wide scope for ethnomedicinal studies as it is inhabited by many tribes. Andrographis paniculata of Assam is used for various treatments by the peoples of different parts of Assam. Phytochemical investigation on ethanolic leaf extract proved the presence of different chemicals in this plant. Thin layer chromatography and IR Spectroscopic studies also confirmed the presence of many chemical compounds in this plant.*

**Keywords:** *Andrographis paniculata*, leaf extract, medicinal.

### Introduction

Assam has an abundance of medicinal plants, against various diseases, known to the native people<sup>1,2</sup>. *Andrographis paniculata* is an important plant of Assam. It is applied for various treatments by the peoples of different parts of Assam. Whole plant is used as an anti diabetic plant by the tribal peoples of Assam<sup>3,4</sup>. It is used by people of upper Assam in killing intestinal worms, in urinary trouble, itching and piles<sup>5,6</sup>. It is also used as hepatoprotective<sup>5</sup>. In upper Assam, leaf juice extract is used to cure irregular bowels in children<sup>7</sup>. Leaf extract is used against common fever by Koch Rajbangshi of Bongaigaon<sup>8</sup>. Leaf and stem juice extract is used in dysentery and stomach trouble in cachar district<sup>9</sup>.

Many research groups in India and other countries in the world have extensively studied the phytochemical compositions of *A. paniculata*; it was observed that the phytochemical compositions of *A. paniculata* are not same in the samples collected from different locations, i.e., phytochemical compositions varies from one location to others.

It is observed that *A. paniculata* mainly contains flavonoids<sup>10</sup>. Presence of flavonoid was reported in the extract of *A. paniculata* collected from most of the location in India like Jaipur<sup>11</sup>, Coimbatore<sup>12,13</sup>, Erode<sup>14</sup>, Vellore<sup>15</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>17,18</sup>, Trichy<sup>19</sup>, Kolli hills<sup>20</sup>, Wandiwash<sup>21</sup>, Raipur<sup>22</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Rampurhat<sup>25</sup>, Basavkalyan<sup>26</sup>, Alappuzha<sup>27</sup>, Orathanadu<sup>28</sup>, Thanjavur<sup>29</sup> and also from Indonesia<sup>30</sup>, Pakistan<sup>31</sup>, Saudi Arabia<sup>32</sup>, Malaysia<sup>33</sup>, and Nigeria<sup>34,35,36</sup>. Alkaloids was reported in the samples collected from Jaipur<sup>11</sup>, Coimbatore<sup>12,13</sup>, Erode<sup>14</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>18</sup>, Trichy<sup>19</sup>, Kolli hills<sup>20</sup>, Wandiwash<sup>21</sup>, Raipur<sup>22</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Rampurhat<sup>25</sup>, Basavkalyan<sup>26</sup>, Alappuzha<sup>27</sup>, Orathanadu<sup>28</sup>, Vellore<sup>37</sup>, Pakistan<sup>31</sup>, Saudi Arabia<sup>32</sup> and Nigeria<sup>34,36</sup>. Similarly, Steroids was reported from the samples

collected from Jaipur<sup>11</sup>, Coimbatore<sup>12,13</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>17,18</sup>, Trichy<sup>19</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Thanjavur<sup>29</sup>, Kanyakumari<sup>38</sup> and Nigeria<sup>34,36</sup>.

Phenolic compounds was confirmed, as reported, in the extracts prepared from the samples collected from Jaipur<sup>11</sup>, Coimbatore<sup>12,13</sup>, Erode<sup>14</sup>, Bangalore<sup>18</sup>, Basavkalyan<sup>26</sup>, Orathanadu<sup>28</sup>, Hyderabad<sup>23</sup>, Thanjavur<sup>29</sup>, Vellore<sup>37</sup>, Pakistan<sup>31</sup>, Saudi Arabia<sup>32</sup>, Malaysia<sup>33</sup> and Nigeria<sup>34,36</sup>. The presence of tannins was revealed, as reported, in the extracts prepared from the samples collected from Jaipur<sup>11</sup>, Coimbatore<sup>12,13</sup>, Erode<sup>14</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>17,18</sup>, Trichy<sup>19</sup>, Wandiwash<sup>21</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Rampurhat<sup>25</sup>, Basavkalyan<sup>26</sup>, Thanjavur<sup>29</sup>, Pakistan<sup>31</sup>, Saudi Arabia<sup>32</sup> and Nigeria<sup>36</sup>.

Saponins was confirmed, as reported, in the extracts prepared from the samples collected from Coimbatore<sup>12,13</sup>, Erode<sup>14</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>18</sup>, Trichy<sup>19</sup>, Kolli hills<sup>20</sup>, Wandiwash<sup>21</sup>, Raipur<sup>22</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Orathanadu<sup>28</sup>, Vellore<sup>37</sup>, Indonesia<sup>30</sup>, Pakistan<sup>31</sup>, Saudi Arabia<sup>32</sup> and Nigeria<sup>34,36</sup>. Glycosides was detected, as reported, in the extracts prepared from the samples collected from Coimbatore<sup>13</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>17,18</sup>, Kolli hills<sup>20</sup>, Raipur<sup>22</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Basavkalyan<sup>26</sup>, Orathanadu<sup>28</sup>, Thanjavur<sup>29</sup>, Indonesia<sup>30</sup>, Pakistan<sup>31</sup>, Saudi Arabia<sup>32</sup> and Nigeria<sup>34,35,36</sup>.

Terpenoids was detected, as reported, in the samples from Erode<sup>14</sup>, Vellore<sup>15</sup>, Bangalore<sup>17</sup>, Trichy<sup>19</sup>, Wandiwash<sup>21</sup>, Raipur<sup>22</sup>, Hyderabad<sup>23</sup>, Basavkalyan<sup>26</sup>, Alappuzha<sup>27</sup>, Orathanadu<sup>28</sup>, Kanyakumari<sup>38</sup>, Indonesia<sup>30</sup>, Saudi Arabia<sup>32</sup>, Malaysia<sup>33</sup> and Nigeria<sup>34,35</sup>. Carbohydrate was present, as reported, in the samples collected from Vellore<sup>15,37</sup>, Chitrakoot<sup>16</sup>, Bangalore<sup>17,18</sup>, Trichy<sup>19</sup>, Kolli hills<sup>20</sup>, Hyderabad<sup>23</sup>, Guntur<sup>24</sup>, Rampurhat<sup>25</sup>, Basavkalyan<sup>26</sup>, Alappuzha<sup>27</sup>, Thanjavur<sup>29</sup> and Nigeria<sup>34,35</sup>.

Protein was reported in the extracts collected from a few areas like Trichy<sup>19</sup>, Bangalore<sup>18</sup>, Kolli hills<sup>20</sup>, Raipur<sup>22</sup>, Hyderabad<sup>23</sup>, Rampurhat<sup>25</sup>, Basavkalyan<sup>26</sup> and Vellore<sup>37</sup>. Similarly, Coumarins was reported in the extracts collected from Trichy<sup>21</sup>, Thanjavur<sup>29</sup> and Kanyakumari<sup>38</sup>.

Anthraquinone, as reported, was present in the samples collected from Bangalore<sup>18</sup>, Hyderabad<sup>23</sup> and Guntur<sup>24</sup>. Oil and fat was reported only in the extracts collected from Alappuzha<sup>27</sup> and Vellore<sup>37</sup>.

Ascorbic acid was reported only in the extracts collected from Basavkalyan<sup>26</sup>. Similarly, resin was reported only in the extract collected from Chitrakoot<sup>16</sup> and lignins were reported only in the extracts collected from Rampurhat<sup>25</sup>.

Here in this report some results of phytochemical and TLC studies on leaf and stem extracts of *Andrographis paniculata* in ethanol is presented.

## Material and methods

**Collection of plant sample:** *Andrographis paniculata*'s plant sample was collected from rural Kamrup of Assam and washed with sterile distilled water; then dried under shaded condition.

**Preparation of plant extract:** In a Soxhlet apparatus, 15gm of the dried cum powdered material (leaf) was continuously extracted with 150 ml of rectified spirit for about 72 hours. The extracted material was then filtered and heated on a water bath before storing in a refrigerator<sup>39,40</sup>.

**Thin layer chromatography:** Silica gel coated air dried micro slides were used as thin layer chromatographic glass plates. The plates were heated inside a hot air oven at 100-120°C to make them moisture free. Samples were spotted carefully on these cooled activated plates using a fine capillary tube, followed by developments with different solvent systems. Spots on these developed glass plates were detected using iodine<sup>39,40</sup>.

**Phytochemical assessment of the extracts:** Different standard procedures were used to detect phytochemicals in the crude extracts<sup>41</sup>. Protein was tested by ninhydrin test, biuret test, lead acetate test, and xanthoprotein tests<sup>42,43</sup>. For detection of carbohydrate Fehling test, Benedict test and iodine test were performed<sup>42,43</sup>. FeCl<sub>3</sub> test, PbAc<sub>2</sub> test, Br<sub>2</sub> water test, acetic acid test and potassium permanganate test was performed for detection of phenols and tannins<sup>42-47</sup>. The presence of flavonoids was tested with pew test, alkaline reagent test, PbAc<sub>2</sub> test and FeCl<sub>3</sub> test<sup>42-47</sup>. Alkaloids were detected by Dragendorff test, Wagner test and Mayer test<sup>42-47</sup>. Liebermann test, Salkowski test, Keller-kilani test and Legal test were performed for detection of glycosides<sup>42-45,47</sup>. Steroid was detected using Liebermann's test<sup>42-46</sup>. Terpenoids were identified by chloroform-sulfuric acid method<sup>42,43,45</sup>. Stable foam formation method was employed for detection of saponins<sup>42-47</sup>.

**FT-IR Spectroscopy:** FT-IR spectra were taken in a Perkin Elmer Spectrophotometer (Version10.4.00) using KBr pellet.

## Results and discussion

*Andrographis paniculata* was tested with thin layer chromatographic as shown in Table-1. In Table-1, it is seen that four spots were obtained with solvent systems S1 (Sl. No. 1), which indicates that the leaf extracts of *Andrographis paniculata* is a complex mixture of chemicals. Hence, the leaf extracts of *Andrographis paniculata* was subjected for phytochemical investigation. Table-2 summarized the Phytochemical investigation reports. Table-2 confirmed that leaf of *Andrographis paniculata* collected from rural Kamrup of Assam composed of phenols and tannins, flavonoids, steroids, terpenoids and alkaloids, on the other hand, leaf extract of *Andrographis paniculata* does not contains proteins, carbohydrates, saponins and glycosides.

In Figure-1, appearance of peaks at 1637.85cm<sup>-1</sup>, 1618.13cm<sup>-1</sup> and 1684.23cm<sup>-1</sup> indicates the appearance of unsaturated bonds as well as carbonyl group. Band around 3417cm<sup>-1</sup> confirms the presence of hydroxyl groups of phenols.

**Table-1:** TLC of leaf extracts.

Solvent System		Extract of <i>Andrographis paniculata</i>	
Composition	Ratio	Number of spots	R <sub>f</sub> values
Hexane : Ethyl acetate (S1)	1:1	4	0.9, 0.7, 0.4, 0.2
Acetone: Ethyl acetate (S2)	1:2	3	0.4, 0.2, 0.1
Ethyl Acetate: Petroleum Ether (S3)	1:9	3	0.9, 0.7, 0.3
Ethyl Acetate: Petroleum Ether (S4)	1:6	2	0.5, 0.4

**Table-2:** Chemical constituents present in leaf extracts [(+) = Present; (-) = absent].

Phytochemicals	Test name/ Reagent	Results
Carbohydrates	Fehling	-
	Benedict	-
	Iodine	-
Proteins	Ninhydrin	-
	Biuret	-
	Lead acetate	-
	Xanthoprotein	-
Phenols and tannins	Ferric chloride	+
	Lead acetate	-
	Bromine water	+
	Acetic acid	+
	Potassium permanganate	+
Flavonoids	Pew	+
	Alkaline reagent	+
	Lead acetate	+
	Ferric chloride	+
Alkaloids	Dragendorff	-
	Wagner	-
	Mayer	+
Glycosides	Liebermann	-
	Salkowski	-
	Keller-kilani	-
	Legal	-
Steroids	Liebermann	+
Terpenoids	Chloroform-sulfuric acid	+
Saponins	Shaking test for foaminess	-

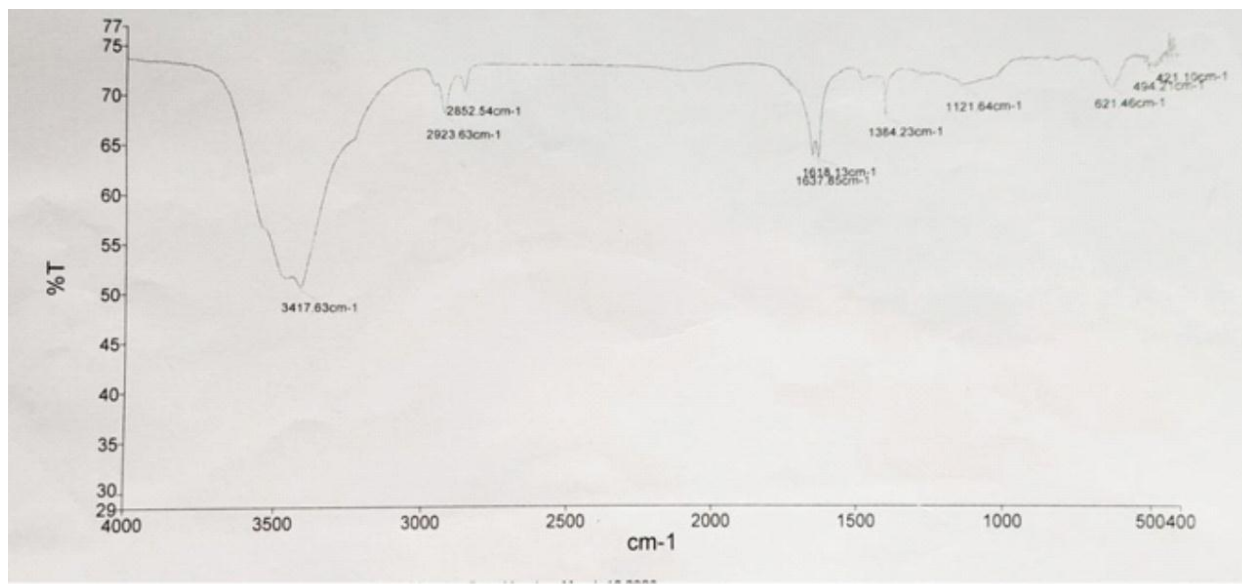


Figure-1: IR spectra of leaf extract of *Andrographis paniculata*.

## Conclusion

*Andrographis paniculata* of Assam is used for various treatments by the peoples of different parts of Assam. Thin layer chromatographic and phytochemical investigation confirmed that *Andrographis paniculata* of rural Kamrup of Assam possesses unique characteristics. Phytochemical investigation on ethanolic leaf extract of *Andrographis paniculata* proves the existence of phenols and tannins, flavonoids, steroids, terpenoids and alkaloids. Again appearance of multiple spots in thin layer chromatographic study confirmed the existence of mixtures of chemicals in the leaf extract of *Andrographis paniculata*.

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