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Gum Arabic

More Than Emulsifier and Food Additive:

A New Experimental Validation

of Anti-Proliferative Against Colorectal

Colon Cancer Cell lines: Part 2

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Abstract

The aim of this paper is to discover the cytotoxicity activity of *Acacia seyal* gum and Prebio-T-commercial (PTC) samples on human colon cancer (HCT-116) cell lines; in addition, its polyphenolic properties are investigated.

Approach

The methodology used in this paper involves fractionating methanol crude extracts of both *Acacia seyal* gum and Prebio-T-commercial, respectively. The cytotoxicity activity on human colon cancer cell lines for each fraction was studied using sulforhodamine B assay (SRB assay). Both samples, *Acacia seyal* gum and Prebio-T-commercial (PTC), were analysed precisely using high performance liquid chromatography (HPLC) technique.

Findings

Based on the analysis, more cytotoxicity activity was identified in the sample obtained from *Acacia seyal* gum (IC_{50}=13.36µg/mL) compared to PTC (IC_{50}=17.18µg/mL). Regarding the flavonoids content (FC), quercetin was the only flavonoids detected in the samples, found to be approximately of 4,640µg/100g for *Acacia seyal* gum, almost double the value of 2,560µg/100g for PTC.

Implications

The implications of this study are the evidence of flavonoids content and anti-cancer activity for both *Acacia seyal* gum and PTC used in folk medicine, as well as anticipation of cancer cell growth. It is expected that the proposed methods will give a noble contribution to the possible use of their bioactive compounds as natural anti-cancer agents.

Keywords

Cytotoxicity activity; *Acacia seyal* gum; Colorectal cancer (HCT-116); Flavonoids, and HPLC.

Introduction

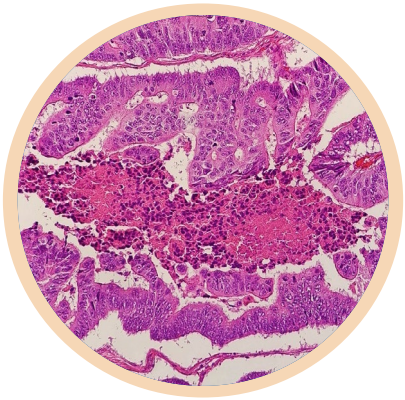
Colorectal adenocarcinoma (HCT-116) cell line is one of the most frequently diagnosed cancers in both males and females globally. The American Cancer Society (ACS) reported about 101,420 new cancer cases, corresponding to 51.3% and 49.7% in males and females respectively, an increase in 2019, whereas there were an estimated 51,020 and 23,380 deaths, males and females respectively, in the USA alone (Siegel et al., 2019). Therefore, more than half (55%) of colorectal cancers in the US are attributable to potentially modifiable risk factors. Unfortunately, accurate statistics on colon and rectal cancer deaths separately are not available because many deaths from rectal cancer are misclassified as colon cancer on death certificates. The World Health Organization (WHO) estimated that 84 million people would die from cancer between 2005 and 2015 (Siegel et al., 2017). This constitutes a severe public health problem in both developed and developing countries.

From a physiological perspective, colon cancer (CC) is one of the most life-threatening diseases where a proliferation of abnormal cells invades and disrupts surrounding tissues (Harlow et al., 2017). The limited success of clinical therapies, including radiation, chemotherapy, immunomodulation, and surgery in the treatment of CC, indicates that there is an imperative need for alternative strategies in CC management (Falk et al., 2016). Clinical treatment remains a challenge, and new natural bioactive compounds are urgently needed. Therefore, the use of natural biological compounds is necessary. For example, the biological activities of such compounds, such as the traditional use of Acacia seyal gum (ASG), are found to be promising.

ASG harvested from tropical plants in the Leguminosae family can be found in particular parts of Ethiopia, Eritrea, Chad, Nigeria, Burkina Faso, Senegal, Mauritania and the primary gum belt in Sudan (Magid et al., 2014). Initially, ASG is reported in various applications in food, nutrition, medicine, dyeing, and cosmetics (Montenegro et al., 2012). ASG was first used in the First World War as solutions injected intravenously as blood treatment of shock and haemorrhage cases among soldiers and other individuals (Bayliss, 1922). Therefore, at the beginning of the 20th century, Europe consumed about 20,000 tons per year of ASG (Chevalier, 1924).

It can be seen that more research should be conducted in a biological aspect using ASG crude extract and their active fractions. Moreover, enriching dietary fibres has led to extensive studies of gum Arabic (GA) as a natural product that has been mainly used as food ingredients and treatment (Phillips et al., 2008). ASG is a rich source of carbohydrates and proteins. It is rich in non-viscous soluble fibres with high dietary value, and minerals, such as potassium, magnesium, and calcium (Al Assaf et al., 2005). According to the Joint Expert Committee for Food Additives (JEFCA) of FAO/WHO, it was defined as “a dried exudation obtained from the branches of *A. senegal* (L) Willdenow or close species from Acacia (Leguminosae family)” (FAO/WHO, 1973). It involves, therefore, both A. senegal and A. seyal species. It was reported that ASG is generally recognised as safe by the Food and Drug Administration (FDA) (Middlekauff, 1975). Therefore, toxicity was not reported in ASG.

The specific objectives of this study were to determine the anti-proliferative property against human breast cancer using methanolic extract and its active fractions.



Material and methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. The HCL, methanol, tert-butyl hydroquinone (TBQH), ascorbic acid and Quercitin were obtained from Merck (Darmstadt, Germany) and RDH (Germany), respectively. Moreover, the HCT-116 cell lines were purchased from American Type Collection Culture ATCC. RPMI 1640 medium, 10% foetal bovine serum (FBS), Streptomycin, Penicillin, CO2, were from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of crude extraction

About 500g powdered sample was extracted with methanol using optimised ultrasonic parameters for 3 hours at a power of 40kHz and 42.5^{0}C. Samples were placed into 20mL vials glass closed tightly for reducing solvent evaporation, following Elnour et al. (2018). With slight modification, methanol was used in the extraction process. The collected solvent of active fraction was concentrated and dried under nitrogen gas flushing at room temperature. However, methanol crude extract (MCE) of *Acacia seyal* gum, subjected to GC-MS/MS to identify the phytochemical compounds, following the method reported by Stankov-Jovanović et al. (2015).

Extraction of Flavonoids

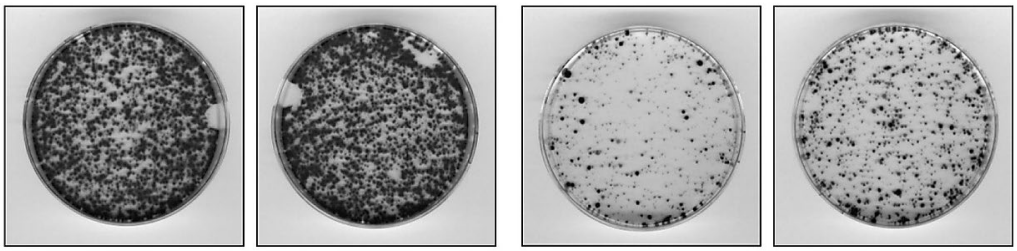
Flavonoid content was determined using Quercetin based on the method of Musaa et al. (2015), with slight modifications. Extraction and hydrolysis procedures were conducted as follows: the sample (0.5g of dried sample) was mixed with 10mL of 1.2 M HCL in aqueous methanol for 3 minutes within a 50mL glass tube at high-performance disperser. A water bath (New Brunswick Scientific, USA) was used to heat the sample mixture at 90˚C for 2 hours with the addition of 10mg TBQH, and at 35˚C for 16 hours with the addition of 8mg ascorbic acid for the phenolic acids hydrolysis. Adjustment with deionised water was made for the extract to reach 20mL, followed by the following stage of cooling down the temperature, and filtration through 0.22µm nylon filter (Whatman, USA) before an injection of extract to HPLC (Shimadzu, Japan). The preparation of the standards was carried out by dissolving them in 0.6 M HCl in 50% aqueous methanol at a range extending from 0.2 to 20mg/mL for calibration curves.

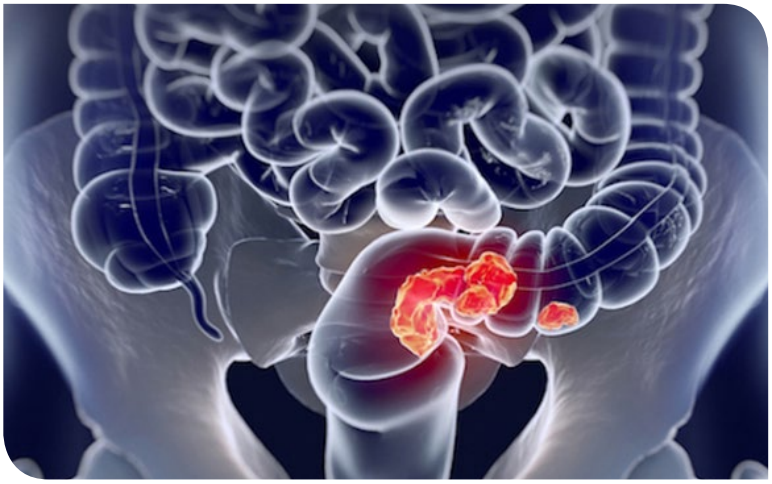
Phytochemical analysis using high performance liquid chromatography

A minor modification was made for the flavonoids analysis that was carried out according to Hayat et al. (2010); the linear AB gradient made up the mobile phase of flavonoids, where 0.1% formic acid formed the solvent A and 100% methanol formed the solvent B, with 0.9mL/min flow rate. The operational column temperature was 40°C with the injection volume set to 20µL. The volume ratios for the solvent gradient were observed at 0-20min, with 55% and 45% solvent A and B, respectively. The quantification of flavonoids compounds was observed at the area of 365nm. All the identified flavonoids compounds were quantified by a standard external method using calibration curves, and their concentrations were expressed as microgram per 100g of dry weight (mg/g DW).

Cell Lines and Culture Conditions

In this research, colon cancer HCT-116 cell lines were used as *in-vitro* experimental cancer cells (ATCC N: HTB-22™). These cells were purchased from American Type Collection Culture ATCC. Frozen HCT-116 cells were thawed and inoculated into 5mL of RPMI 1640 medium, enhanced with 10% foetal bovine serum (FBS) and supplemented with 100µg/mL streptomycin and 100U/mL penicillin. The HCT-116 cells were cultured in T-25 flasks and incubated at 37°C in 95% humidified incubator with 5% CO_2. When they reached 70% confluency, the cells were used for further experiments.





Anti-proliferative effect of *Acacia seyal* gum

In the *in-vitro*, cytotoxic activity of the MCE and its active fractions against MCF-7 cell lines were determined using sulforhodamine assay (SRB assay), as previously described by Samarakoon et al. (2016); however, a slight modification was made to the procedure. Briefly, cells were trypsinised and inoculated (5x103 cells/well) into 96-multiwell plates then incubated for 24 hours. After that, different concentrations of the compound under test (0, 1, 2.5, 5 and 10µg/mL) were administered to the cell wells and incubated for 48 hours. Moreover, Taxol was used as a positive control, and DMSO at (01%v/v) served as a negative control. The cells were placed on the ice-cube with 50% trichloroacetic acid solution and incubated for 60 minutes at 4°C, then washed five times with tap water before marking them with sulforhodamine-B stain. After that, cells were stained with 100µL of 0.4% SRB solution/well for 15 minutes at room temperature. The acetic acid solution (1%) was used to remove the unbound dye, and then unbuffered. On the other hand, Tris-based solution was utilised to solubilise the bound SRB dye. Finally, the plates were shaken for 1 hour at room temperature then the readings were recorded by a microplate reader at a wavelength of 540nm. The obtained results were used to calculate the percentage of cell viability, and 50% of inhibitory concentration (IC_{50}) of each extract was determined, using equation 1 (Vichai and Kirtikara, 2006) as follows:

$$\%\ cell\ viability = \frac{mean\ OD\ of\ extract\ wells}{mean\ OD\ of\ control\ wells} \times 100 \qquad \text{(Eq1)}$$

Statistical analysis

The cell viability calculation was performed in triplicate. Each resulting point indicates the overall average of at least three independent trials. The results were examined and expressed in terms of the mean of the samples as well as the standard deviation. GraphPad Prism Version 7.00 (Inc., La Jolla, CA, USA) and Minitab Software version 17® were used to calculate the statistical parameters. Finally, one-way ANOVA and Dunnett's t-test were used to identify any significant differences between the means of several independent samples.

Results and Discussion

Anti-proliferative effect of methanol extract of *Acacia seyal* gum studied

Figures 1 and 2 present the results of anti-proliferative activity (APA) obtained with the MCE of *Acacia seyal* gum (ASG) and PTC respectively, against human tumours cell lines. To our best knowledge, no study has been conducted before regarding APA of *Acacia seyal* gum using MCE on cell lines bases.

In this study, the human colorectal adenocarcinoma (HCT-116) cell lines have been comprehensively studied, and the results are shown in Figures 1 and 2, respectively. Figures 1 and 2 revealed an active crude of methanol, as well as its active bioactive compounds derived from both ASG and PTC samples; these presented cytotoxic activity against HTC-116 cell lines respectively.

In previous studies, the persuasive APA of MCE was reported by several researchers. For example, Jambunathan et al. (2014) investigated rhizomes of *C. Amada* on MCF-7; Kumar et al. (2017) and Chon et al. (2009) studied the effect of the mulberry plant on HCT-116; and Savietto et al. (2013) reported the effectiveness of Croton (*C. erythroxyloides*) on PC3, using 3-(4,5-dimethylthiazol-2-yr)-2,5-diphenyl-tetrazolium bromide (MTT) assay, as well as Sulforhodamine B (SRB). To date, however, no study has been conducted on cell lines using *Acacia seyal* gum in term of raw or extracted form.

However, in this experiment, the cytotoxicity of MeOH crude extract of ASG and PTC, including their bioactive compounds (Quercetin) of ASG and PTC respectively, were evaluated against (HCT-116) cell lines using Sulforhodamine B (SRB) assay. The SRB is the most common method for measuring the inhibition of cell proliferation test as the viral strain.

In this experiment, the SRB assay was used; in addition, the National Cancer Institute criteria (NCIC) was also used, as mentioned by Fouché et al. (2008). The NCIC looks through the mean value of the logarithm of growth inhibition (GI_{50}) for all the tumour cell lines. Based on the NCIC, the MCE obtained from ASG and PTC respectively, showed an average of moderate activity mean of (log GI_{50}=1.00) against cancer cells. This extract also presented a high selectivity for HCT-116 (colon cancer, mean log GI_{50}=0.9805 and 0.994 respectively against HCT-116 cell lines). The NCI criteria show that individual growth inhibition GI_{50} values indicate potent activity (log GI_{50}<0), similar to Taxol. Interestingly, the MCE of the ASG sample was found to be the most active against HCT-116 cell lines, which obtained approximately (13.36µg/mL). This is higher compared to the mean value of IC_{50} = 17.18 µg/mL for PTC against HCT-116 cell lines (Figure 1). The highest growth inhibition of HCT-116 cell lines was, therefore, more potent when using ASG MeOH crude rather than PTC. There are no previous results in terms of using gum arabic extract as a treatment for colon cancer.

Manthey and Guthrie (2002) reported that the activity of extracts with GI_{50} values lower than 20µg/mL should be regarded as secure. Considering this new perspective, both sample extracts of methanolic extract of ASG and PTC showed significant activity against the cell strains analysed (Figures 1 and 2). Therefore, inhibition growth IG_{50} is an advantageous measurement when being recommended by the American National Institute of Cancer.

Nevertheless, some researchers have used different parameters. For example, Boyd (1997) claimed that medicinal plant extract is usually valued as significant for *in-vitro* cytotoxic activity when the IG_{50} value is <100µg/mL. In this regard, our findings indicated that the methanol extracts crude extracts of ASG showed the best cytotoxic activity against the HCT cell lines (Figure 1). Therefore, gum arabic is more than an emulsifier of food additive (E414) as many people thought.

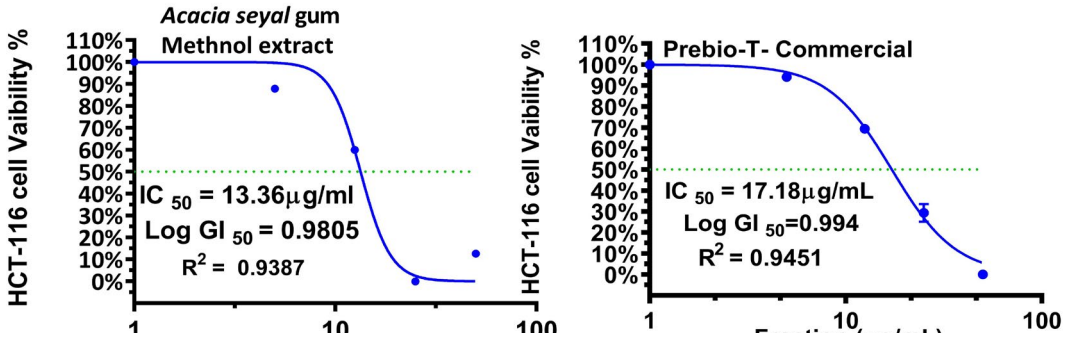


Figure 1: The fitted curve generated for *Acacia seyal* gum and PTC methanolic crude extracts, and their active fractions against Human Tumour Carcinoma (HTC-116) cell lines. Data are based on triplicate experimental sets (N=3)

Source: Devised by authors

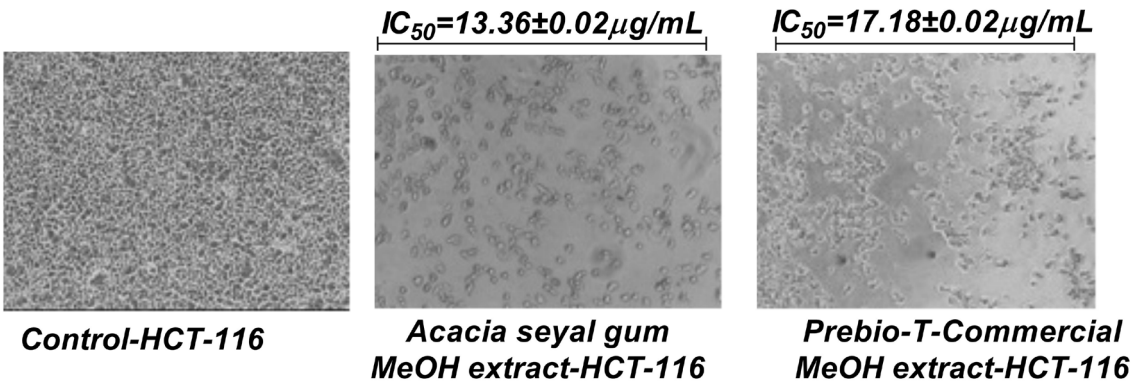


Figure 2: Demonstrative images show surviving HCT-116 cell lines, at 24 hours incubation following the treatment of ASG and PTC crude extract from methanol, and its active fraction/s respectively.

Source: Devised by authors

Determination of flavonoids content

Between the ASG and PTC extracts tested, both samples showed significant amounts of flavanols quercetin while kaempferol, myricetin and apigenin were not detected (Table 1). The flavonoid content of the plant extracts tested in this research were not detected in the earlier literature. The results showed that the quercetin values in the *Acacia seyal* gum were significantly ($p \leq 0.5$) higher (6540±3.46µg/100g) compared to PTC samples. In contrast, the quercetin values of PTC were found to be 2560±3.49µg/100g. The mechanical process hugely affected the flavonoids content in gum arabic (GA). There are no previous studies regarding flavonoids extraction and determination from GA, and the main challenges in extracting and fractionation of polyphenolic compounds from GA are still not understood (Sanchez et al., 2018). Therefore, an investigation into the role of quercetin on the inhibition of cancer is urgently needed.

Moreover, quercetin (only flavonoids detected in both ASG and PTC) for example, was the first tyrosine kinase inhibiting compound tested in a human phase II trial (Anderson and Jankowski, 2003). Heat shock proteins form a complex with mutant p53, which allows tumour cells to bypass standard mechanisms of cell cycle arrest (MCCA). Moreover, therefore, heat shock proteins also allow for improved cancer cell survival under different bodily stresses.

Flavonoids are known to inhibit the production of heat shock proteins in several malignant cell lines, including breast cancer (MCF-7), leukaemia, and colon cancer (Davis et al., 2000). Interestingly, in this study, the authors believed that the flavonoids in ASG extract were not thought to be responsible for inhibiting MCF-7 cells lines: therefore, more investigation will also be needed.

In a similar study, it was shown that the flavanol epigallocatechin-3-gallate inhibited fatty acid synthase (FAS) activity and lipogenesis in prostate cancer cells, an effect that is strongly associated with growth arrest and cell death (Brusselmans et al., 2005). In comparison with most normal tissues, expression of FAS is remarkably increased in various human cancers. Upregulation of FAS occurs early in tumour development and is further enhanced in more advanced tumours (Brusselmans et al., 2003).

In the present study, the quercetin has revealed the double values in the sample (Table 1). Of those, quercetin is known to produce cell cycle arrest (MCCA) in proliferating lymphoid cells (PLC). In addition to its antineoplastic activity, quercetin exerted growth-inhibitory effects on several malignant tumour cell lines *in-vitro*. These included P388 leukaemia cells, gastric cancer cells (NKN-7, HGC-27, NUGC-2, and MKN 28), colon cancer cells (COLON 320 DM), human breast cancer cells, human squamous and gliosarcoma cells, and ovarian cancer cells (Davis et al., 2000). Therefore, gum arabic is more than an emulsifier of food additives (E414) as many people thought. The flavonoids content is as presented in Table 1.

Table 1:
The flavonoids content of *Acacia seyal* gum and Prebio-C in µg/100g

Flavonoids	*Acacia seyal* gum	Prebio-T- commercial
Quercetin	4650 ±4.04[a]	2560 ±3.49[b]
Myricetin	Nd	Nd
Apigenin	Nd	Nd
Kaempferol	Nd	Nd

*Mean value± standard deviation in raw, followed by different superscript letters are significantly different ($p \leq 0.05$). Small superscript letters show the significance when compared between rows. Prebio-T: *Acacia sengal* gum (Commercial). ND: Not detected.

Source: Devised by authors

Conclusions

In summary, *Acacia seyal* gum (ASG) extract exhibits anti-proliferative effects against HCT-116 colon cancer cell lines by inducing loss of cell viability via inhibition and morphology changes. This inhibition was selective to the growth of HCT-11 6, suggesting that ASG extract possesses selective anti-tumour properties towards cancer cells. It also demonstrated their potentiality as an anti-cancer agent when compared with Taxol as a positive control. Furthermore, ASG treatment has inhibited colon cancer HCT-116 cell growth by reducing the number of cell generations and increasing the doubling time compared with the control. These data propose that ASG can be developed as a novel mechanism-based supplement agent for the cure and prevention of colon cancer. More studies are urgently needed to elucidate the mechanisms underlying the therapeutic effects of the ASG extract, as well as an investigation of the active compounds responsible for cytotoxicity.

Conflict of interest

There are no conflicts of interest.

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Biography

Dr Ahmed A.M. Elnour has a BSc in Biochemistry and Food Science from the University of Kordofan (2003), an MSc in Gum Chemistry from the University of Khartoum, Sudan, and a PhD in Biotechnology Engineering from IIUM, Faculty of Engineering. He was previously a Head of the Department of Biochemistry and Gum Processing at the Institute of Gum Arabic & Desertification Studies, University of Kordofan, Sudan. He is a visiting lecturer of Biochemistry and Molecular biology at Western Kordofan University, Faculty of Veterinary Science, Sudan. He is the author or co-author of numerous publications, and has several publications and patents under review in high impact factor journals. https://scholar.google.com/citations?user=undo98wAAAAJ&hl=en

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Prof. Dr Md Z. Alam is a Professor and Chair of BERC, Faculty of Engineering at IIUM, and was Head of the Department of Biotechnology Engineering from 2009 to 2012. He received his BSc in Chemical Engineering from Bangladesh University in 1996, and obtained an MSc in Chemical Engineering from UKM in 1999. He earned a PhD in Biochemical Engineering from UPM in 2002. Professor Alam has 20 years of teaching and research experience, and has authored and co-authored some 500 publications. With the high quality of the journal papers, a large number of citations (about 3,000) are being cited every year with high h-index, 31 at Scopus Database: https://scholar.google.com/citations?user=0FUVN0AAAAJ&hl=en

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